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THE OPTIMAL WATER REQUIREMENT IN RENAL FUNCTION

I. Measurements of Water Drinking by Rats According to Increments of Urea and of Several Salts in the Food

J. L. GAMBLE, M. C. PUTNAM AND C. F. McKHANN

From the Department of Pediatrics, Harvard Medical School

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The terms of the "modern" theory of renal function, as presented by Cushny (1), suggest an attractively simple explanation and definition of the water requirement. According to this theory, as glomerular filtrate passes along the renal tubules water and materials are reabsorbed from it by the tubule cells in such quantities as will sustain the normal composition of the blood plasma. If, as is usually the case, relatively more of water than of substances must be returned to the plasma, reabsorption is accomplished against a rising osmotic pressure in the tubular fluid. It is an easy surmise that, if circumstances require excretion of a large amount of substances with relatively little water, a level of osmotic pressure may be reached which will prevent further withdrawal of water from the tubular fluid, with the result that urine of the maximal possible concentration is produced. Obviously there is here the important implication that the minimal water requirement for excretion of the various substances in urine must be, per molecular amount, the same for each of them. This hypothesis agrees with Ambard's conception of an identical "volume obligatoire" for the individual substances based on an approximate equivalence of the highest concentrations of urea, of glucose and of sodium chloride observed in urine. Ambard's data were not extensive nor was their agreement of a convincing degree. Although subsequent investigations of this point have been numerous, information of a satisfactory precision is still lacking. It may be said however, in brief appraisement, of the evidence at hand that roughly the same values for maximal concentration in urine have been found for NaCl and $NaHCO_3$, and that this value probably also obtains for a mixture of these salts (2), that maximal concentrations determined for urea are in average somewhat higher than is found for NaCl (3), that urea and NaCl

together in various proportions produce curiously differing maximal values (4).

But it must be admitted that the upper limits of concentration for individual substances in urine have not been dependably established. This desirable datum has been found surprisingly elusive. As an initial obstacle, it is impossible to provide conditions which will produce urine of maximal concentration containing only one substance. All that can be undertaken is to make the substance which is being studied as large as possible a factor in the total quantity of material claiming excretion in the urine. Water must be restricted with the hope of supplying less than the minimal requirement. Attempts in quest of maximal concentration values in the urine have consisted in intravenous injection of heavily hypertonic solutions of individual substances or else ingestion of large amounts of such solution. Successive voidings of urine are then collected, concentration measurements obtained and the highest value found is taken as maximal. There are obvious objections to this plan of study. It must place regulatory mechanisms in the body fluids under sudden and severe stress. The intravenous injection of solutions of a single substance, for instance, may be expected to greatly distort the normal chemical anatomy of the blood plasma. Moreover, since less than the minimal requirement for water is supplied, the deficit must be obtained from the body fluids. Evidently such an experiment places the organism in more or less severely abnormal circumstances which quite probably may interfere with the attempt to measure the extent of a normal process in the kidney. Addis (5), reviewing the results of such studies, concludes that even the idea that there is a limit to the kidneys' ability to concentrate a substance in urine remains an unestablished concept. He points out that at the limit of possible conditions as regards restriction of water and increase of substance, the successive values for concentration found in the urine lie along a curve which continues to ascend until the animal dies as a result of the severity of the experimental plan; and up to this point the kidney continues to excrete increasing amounts of the substance smoothly and accurately in terms of the mounting concentration in the plasma. There is thus obvious naiveté in the assumption that the final measurement of concentration marks the limit of an intrinsically renal process.

The considerations above presented suggest the necessity and, perhaps, the desirabilty of somewhat altering our conception of objective in studying the water requirement in renal function. It would seem probable that measurements of optimal concentrations of substances in urine under experimental conditions permitting the organism to establish a steady state, if such data can be obtained, might provide more dependable and possibly more cogent information than is likely to be gained by determination of dubiously maximal concentrations in the presence of abnormal circum-

stances. In other words, instead of attempting to define the extensibility of a single governing factor in the water requirement, it might be more profitable to undertake to learn the quantity of water which it best suits the organism to use in accomplishing the conveyance and excretion in urine of a given amount of an individual substance. The experiments described in this paper represent an attempt in this direction.

PLAN OF EXPERIMENTS. Rats were placed on diets containing successive increments of a single substance, or of a mixture of substances, and daily measurements made of food eaten and of water drunk. The substances used were urea, NaCl, KCl, and $KHCO_3$. The experiments thus consisted in depending upon the rats to extend their intake of water accurately according to additions of these materials to the diet. Obviously this extremely simple plan of study will not directly measure the quantity of water required for removal of the added substance in urine. Assuming correct drinking by the rats, it should however provide comparative data of an accuracy sufficient to indicate appreciable differences, if any, in the individual requirements of the several substances studied. The experiments were undertaken on the basis of this expectation. The ubiquitous obstacle, consisting in materials other than the substance studied claiming excretion in urine, was present in these experiments. The rats, however, were willing to eat diets containing such enormous additions of salt or urea that the theoretical goal of a urine containing a single substance was fairly closely approached. A chief feature of the experiments was their duration. The data obtained for each step in the addition of a substance to the diet represent a period of one week. They can therefore be regarded as statistical and also as the product of a thoroughly established steady state. Moreover the quantity of the substance which accumulates in the body fluids in order to provide a concentration sufficient to establish the required rate of excretion is a negligible part of the total intake for one week. This troublesome factor in experiments based on a single ingestion of a substance is thus avoided.

The diets used were of the so-called purified type. The method of their construction, in all of the experiments, is illustrated by the diagrams in the lower part of figure 1 which describes a sodium chloride and a urea experiment. The composition of the basal diet is shown by the first column of the diagrams. The essential point is that it contains protein and salt mixture in amounts minimal for satisfactory nutrition in order that the quantity of material to be excreted in urine, besides the added single substance, be kept as low as possible.[1] Each of the steps in the addition of sodium

[1] Basal diet, per cent composition:

Casein	12
Salt mixture	3
Yeast	3

chloride or of urea represent one millimol per gram of food. In the case of the salt, the millimol is an "osmolar" one; that is, each increment was a half millimol of molecular sodium chloride, on the assumption of its practically complete ionization in urine. It was considered a desirable condition of the experiments that increase of rate of excretion of the added substance correspond fairly closely with the increments per gram of food. It was therefore necessary that the rats eat approximately the same quantity of food during the successive periods. To this end, as shown in the diagrams, with each dilution of energy content by addition of salt or urea, fat was increased and starch reduced to the extent necessary to provide a stationary caloric value per gram.[2] The rats used in all of the experiments were young adult males of the same age (±5 days) and approximately the same weight. The data from each experiment were produced by a pair of animals placed together in a small circular cage containing a food cup with funnel-shaped inlet to prevent spilling and a drinking tube fashioned from a 100 cc. Shellbach burette. Measurements of food eaten and water drunk were made daily. As already mentioned the individual periods of an experiment were each of a week's duration.

Starch 70
Milk fat 12

The salt mixture was composed of the following substances which were in the amounts given, ground very thoroughly together in a mortar:

	gm.
$CaCO_3$	32.5
$3\,MgCO_3Mg(OH)_2 \cdot 3H_2O$	7.6
KCl	27.5
$K_3C_6H_5O_7 \cdot H_2O$	5.5
$NaH_2PO_4 \cdot H_2O$	25.8
Na_2CO_3	5.1
$Fe_2(NH_4)_2(C_6H_5O_7)_2 \cdot 3H_2O$	1.0

The materials from the basal diet claiming excretion in urine compose a value of about 1.0 mM. per gram of food. This is a rough approximation arrived at by calculating the urea equivalence of nitrogen from the casein intake, which amounts to 0.68 mM. per gram of food, and adding a value for the salt mixture of 0.37 mM. per gram of food obtained by computing the sum of the millimolar quantities of Mg, K, Na, Cl, and P; Ca and Fe, on the ground that they do not to an appreciable extent enter the urine, being omitted.

We are indebted to the courtesy of the Northwestern Yeast Company for a generous supply of the dried yeast used in these experiments.

[2] The water of oxidation from a gram of food is only slightly altered by this adjustment of the fat and starch factors; the increase which additions of fat tend to produce being slightly more than offset by the reduction in the sum of the two factors. The value for water of oxidation from the basal diet is 0.520 cc. per gram. For the last period of salt addition in the NaCl experiment, this value is 0.486 cc., or 0.034 cc. per gram less than during the fore period. The corresponding reduction in the urea experiment is 0.057 cc. per gram. Evidently differences of this degree in the amount of water from oxidations will only slightly disturb the relationship of the water intake to the additions of NaCl or urea to the diets.

THE WATER REQUIREMENT FOR SODIUM CHLORIDE AND FOR UREA. The direct measurements obtained from a sodium chloride and from a urea experiment are given in table 1. These data are from two pairs of rats of identical average body weight at the outset of the experiments. Although it may be said that the diets were surprisingly well taken there was, as may be seen in the table, appreciable decline in the daily quantity of food eaten and depression of the rate of body weight gain, these effects being more evident in the urea experiment. There was no change in the behavior of the animals nor was there any visible evidence of edema. The extension of water drinking was quite remarkable, the intake per day during the last period of the sodium chloride experiment being equivalent to one-half of the body weight.

TABLE 1

PERIODS, 1 WEEK EACH	NaCl EXPERIMENT				UREA EXPERIMENT			
	NaCl added	Body weight	Food eaten	Water drunk	Urea added	Body weight	Food eaten	Water drunk
	per cent	*grams*	*grams*	*cc.*	*per cent*	*grams*	*grams*	*cc.*
I	0.0	205	13.2	17.2	0.0	205	13.2	12.7
II	2.9	215	13.8	28.8	6.0	212	12.3	16.2
III	5.8	218	12.3	40.3	12.0	213	11.0	21.3
IV	8.7	224	13.0	58.0	18.0	219	11.7	33.5
V	11.6	226	11.8	73.0	24.0	216	10.5	41.7
VI	14.5	227	12.4	100.0	30.0	218	11.7	59.8
VII	17.4	232	12.1	118.0	36.0	216	10.4	65.2
VIII	0.0	255	12.8	21.1	0.0	232	12.7	14.3

The measurements of body weight are one-half the weight of the pair of rats at the end of the period. The figures for food eaten and water drunk are average daily values for a single animal obtained by dividing the quantities found for each 7-day period by 14.

The relationship of water intake to the quantity of material requiring excretion in urine is graphically described in the upper part of figure 1. The horizontal lines record on the left hand ordinate the cubic centimeters of water drunk per gram of food eaten. The points connected by broken lines represent concentration of NaCl or of urea in terms of the water intake. These values are obtained by dividing the number of millimols of added substance per gram of food (given at the bottom of the figure) by the just mentioned datum; the cubic centimeters of water drunk per gram of food eaten. The molar concentrations thus obtained may be read on the right hand ordinate. These are of course not to be taken as the actual concentration of NaCl or of urea in the urine. The urine values will be somewhat higher and moreover will not quite parallel these data since the

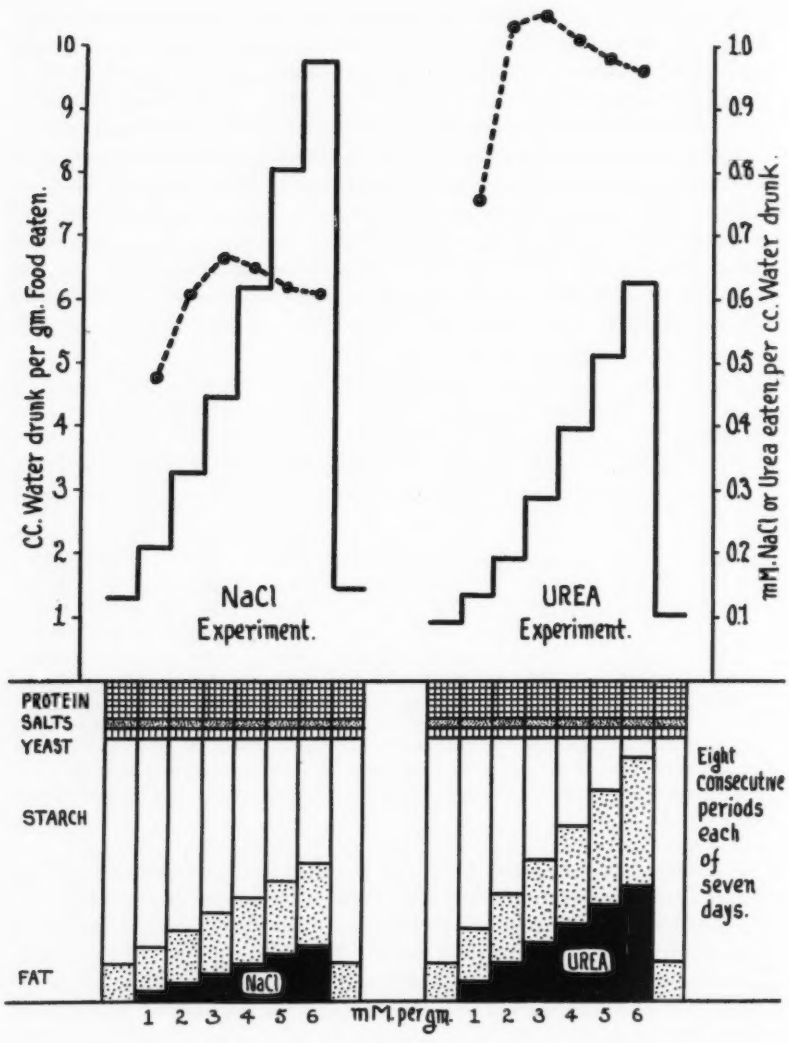

Fig. 1

quantity of water leaving the body by way of the lungs and skin, having presumably a stationary value, becomes a diminishing fraction of the water intake in the successive periods of the experiments.

It may be noted that in both experiments at the upper levels of added substance the steps in extension of water drinking are fairly regular, those produced by adding sodium chloride to the food being however much larger than for corresponding increments of urea. When two millimols or more of substance per gram are added an approximately stationary concen-

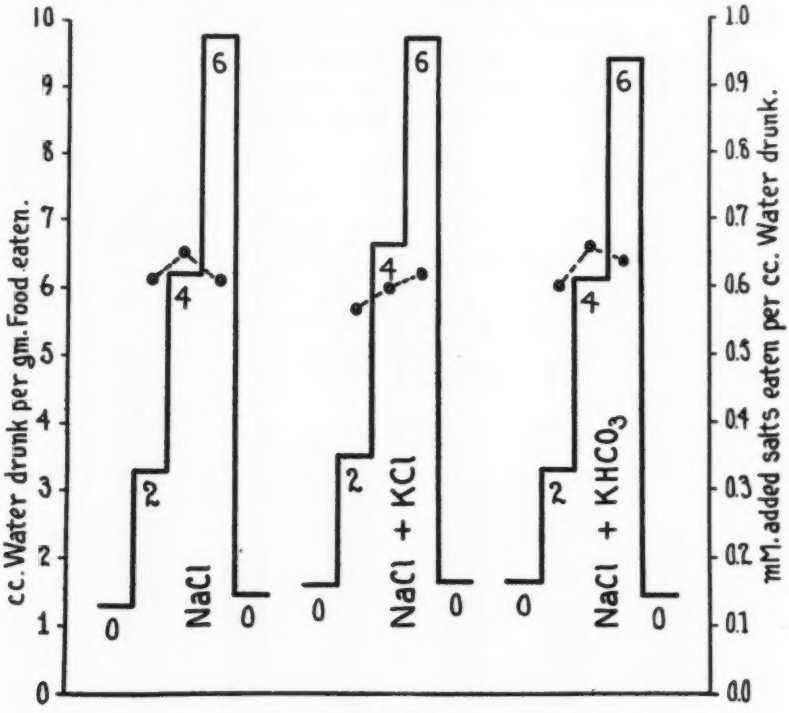

Fig. 2

tration in terms of water intake is established; the values found for sodium chloride lying near an average of 0.63 M. and those for urea being close to an average of 1.01 M.[3]

[3] It is of some interest to compare these values for concentration of ingested NaCl or urea in terms of an optimal water intake found for the rat with those for "maximal" concentrations of these substances determined directly in human urine. Adolph (3), by means of ingestion experiments carried out on himself, found for NaCl, assuming here complete ionization, an average maximal value of 0.60 M. For urea the maximal concentrations found differed considerably, their average value being 0.71 M. Ambard's "highest" concentrations were 0.73 M. for NaCl, and 0.93 M. for urea.

A curious detail of these data is that the highest concentration value was in both experiments obtained when the food contained 3 millimols of substance per gram, further additions causing a small but definite and progressive decline. Perhaps this finding represents reduction of the usual extent of water reabsorption as a result of the increasing volume and rapidity of flow of tubular fluid; the hypothetical event described as tubule diuresis. The simplicity of this explanation is however disturbed by the

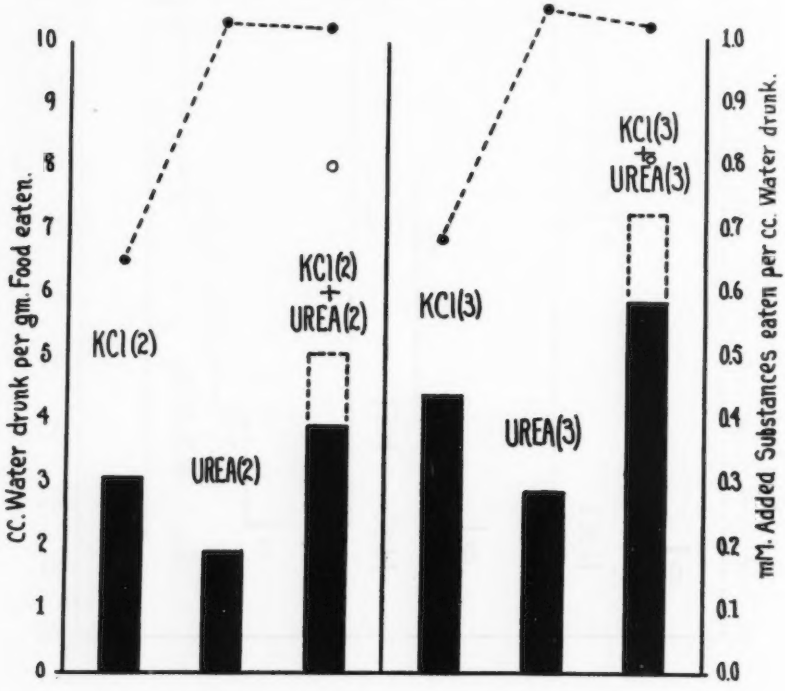

Fig. 3

fact that the rate of production of tubular fluid is much less for urea than for an equivalent amount of NaCl.

It is clearly the evidence of these experiments that the optimal requirement for water for removal of sodium chloride in urine is much greater than for an equivalent amount of urea.

THE WATER REQUIREMENT FOR MIXTURES OF SALTS. In figure 2 the data obtained by additions to the basal diet of mixtures of NaCl and KCl, and of NaCl and $KHCO_3$, are compared with those found for corresponding

amounts of NaCl alone. The steps in these experiments are of one millimol (osmolar) of each of the two added substances, providing in three successive periods intakes of 2, 4 and 6 millimols of added salts per gram of food. As may be seen in the figure, the levels of water drinking for the increments of the salt mixtures and also the concentration values in terms of the water intake agree closely with those found in the sodium chloride experiment. It is therefore evident that the optimal water requirements for removal of each of these several salts in urine are identical or at least nearly so. Since this finding was obtained by using mixtures of salts, the additional information that the individual requirements are, at least closely, additive is produced.

THE WATER REQUIREMENT FOR MIXTURES OF UREA AND SALTS. The fact that the optimal water requirements for removal of salts and of urea differ widely did not disturb an expectation that they would be found to be additive. The results of two experiments undertaken to test this point are

TABLE 2

ADDITIONS OF KCl OR OF UREA TO BASAL DIET	WATER INTAKE PER GRAM FOOD INGESTED	SALT OR UREA INGESTED PER CC. WATER INTAKE
mM. per gram	*cc.*	*mM.*
KCl, 2	3.09	0.65
Urea, 2	1.94	1.03
KCl, 2 + Urea, 2	3.90	1.02
KCl, 3	4.38	0.69
Urea, 3	2.86	1.05
KCl, 3 + Urea, 3	5.83	1.02

presented by means of the diagrams in figure 3. The first column in the first section of the figure represents the water intake per gram of food containing 2 mM. KCl. The next column measures the water intake on a diet containing 2 mM. urea per gram. The extent of water drinking when the diet contained 4 mM. of added material composed of 2 mM. KCl and 2 mM. of urea is shown by the third column. The expected height of this column, on the assumption that the individual requirements are additive, is shown by broken lines and the expected concentration of the mixture of substances referred to water intake is indicated by a circle. The actual height is interestingly just double that of the preceding column with the result that the concentration of the two substances taken together, referred to water intake, is the same as found when urea alone is added to the food. Repeating the experiment at a higher level of added substance gave, as may be seen in the second half of the figure, identical results.

The data from which these diagrams were constructed are given in table 2 and from them a descriptive explanation of this curious finding can be

easily derived. The concentration found for KCl + urea is 1.02 M., approximately the value for urea alone. Since these two substances are present in equal amounts, their concentrations, referred individually to the water intake, are 0.51 M. This value lies below that for additions of KCl alone to the diet, which according to these data is about 0.67 M. There is thus here temptation for the generalization that urea and salts may be excreted together in urine at the concentration possible for urea alone provided the level permitted for salts is not exceeded by their partial concentration.

SUMMARY

The two questions of whether or not the optimal water requirement for removal in urine of urea and of several salts (NaCl, KCl and $KHCO_3$) is the same for each of these substances and additive for mixtures of them, was studied in a series of experiments by measuring the water intake of young adult rats on a basal diet to which were added successive increments of urea and of salts, singly and together.

The data obtained demonstrate: 1, that the optimal water requirement for each of the salts is identical, or nearly so, and that the individual requirements are, at least closely, additive; 2, that the quantity of water required for removal of urea is much less than for corresponding amounts (osmolar) of the salts studied; 3, that the differing requirements for urea and salts are not additive; the requirement for a mixture of equal amounts of urea and of salt being the same as for an equivalent quantity of urea alone.

BIBLIOGRAPHY

(1) Cushny, A. R. The secretion of urine, 2nd ed., London, 1926.
(2) Davies, H. W., J. B. S. Haldane and G. L. Peskett. Journ. Physiol., 1922, lvi, 269.
(3) Adolph, E. F. This Journal, 1923, lxv, 419.
(4) Chaussin, J. Journ. Physiol. Path. gén., 1920, xviii, 895.
(5) Addis, T. and M. A. Foster. Arch. Int. Med., 1924, xxxiv, 462.

THE RÔLE OF FIBER SIZE IN THE ESTABLISHMENT OF A NERVE BLOCK BY PRESSURE[1] OR COCAINE

HERBERT S. GASSER AND JOSEPH ERLANGER

From the Departments of Pharmacology and Physiology of the Washington University School of Medicine

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A review of the literature on depression of conduction in a nerve trunk reveals the fact that there is a more or less definite order in the susceptibilities of the various fibers. In a cocainized nerve, sensory fibers are described as being more easily depressed than motor fibers, and as having different thresholds among themselves, so that the time of block depends upon the functions mediated. Although the data on this subject are not in complete accord, the order of disappearance which receives the strongest support is, beginning with the most susceptible: pain, cold, warmth and contact. This is the order which was observed by Baglioni and Pilotti (1909) and later by Thöle (1912) to obtain in spinal anesthesia, a condition which is really induced by a perineural application of the anesthetic. That cold is blocked before warmth was also the finding of Ponzo (1908), of Schilder (1913) and of Goldscheider and Hahn (1924); and Bier (1899) described pain as going before temperature and contact. On the other hand, Goldscheider (1886) observed temperature to disappear before pain in the distribution of a cocainized nerve branch.

While cocaine has been most studied, its mode of action is not a feature of cocaine alone. Similar effects have been described for the aliphatic narcotics, phenols, aconitine, etc.; and these effects have therefore been associated with the nature of the nerve fibers themselves. No experimental data have been presented, however, to indicate what this quality of the nerve fibers might be. Cushny in his textbook states that no explanation of the difference between the responses of sensory and motor nerves is known. On the other hand the assumption is widely made (e.g., Gottlieb in Meyer and Gottlieb's text, and Poulsson in Heffter's *Handbuch der Pharmakologie*) that the phenomenon is due to a difference in chemical constitution with a corresponding difference in affinity. But when we see the same order, only reversed, emerging from the data on compression blocks, it is apparent that the postulate of different chemical affinities is not a sufficient explanation.

[1] A preliminary report on the effect of pressure was published in the Proc. Soc. Exp. Biol. and Med., 1927, xxiv, 313.

The data for compression are more fragmentary and less concordant than those for cocaine; but there is evidence that motor fibers are blocked before sensory fibers and that sensations disappear in the order: contact, cold, warmth, pain. The relationship between the susceptibilities of motor and sensory nerves was determined nearly half a century ago by Luederitz (1881). He was stimulated to perform his experiments by the clinical observation that, following mechanical lesions of mixed nerves, motility is more injured than sensation; and he succeeded in duplicating the clinical picture in the sciatic nerve of the rabbit. Although his conclusion—namely, that motor fibers are more susceptible than sensory fibers—has not been confirmed either by Zederbaum (1883), Efron (1885), or Ducchesi (1901), the experience obtained in the present research indicates that it is essentially correct. The order designated for the failure of conduction of the various kinds of sensory impulses has been derived from the observations of a number of experimenters. In 1885 Herzen showed that pressure on nerves blocks contact and cold before warmth and pain. In the following year Goldscheider (1886) confirmed the blocking of cold before warmth; but he found that contact, although affected simultaneously with cold, persisted longer so that, with complete anesthesia to the latter, contact and warmth, though subdued, could be felt. More recently Fabritius and Bermann (1913) made the observation that, when the last trace of the contact sense had gone, pain and temperature (including cold and warmth) could still be felt, the pain being very definite.

It is to be expected that the solution of the problem of the mechanism of the differential susceptibility of nerve fibers is to be found in some quality of nerves which varies from fiber to fiber, and this at once suggests a survey of the known characteristics of nerve fibers in search of such a factor. The electrical responses in individual fibers—that is, the axon potentials—are the same in all medullated fibers larger than 5μ (Erlanger, Bishop and Gasser, 1926; Gasser and Erlanger, 1927), and possibly also in some fibers smaller than this; so too are the refractory phases (Erlanger, Gasser and Bishop, 1927): points strongly suggesting that the fundamental chemical or physico-chemical constitution of nerve fibers is the same in all above this size. What varies is the threshold of excitation and the velocity of conduction; but these in turn are dependent upon the fiber size (Lapicque and Legendre, 1913, 1922; Lapicque, Gasser and Desoille, 1925; Gasser and Erlanger, 1927). Thus the physiology of nerve strongly suggests that fiber size should be an important factor in the resistance of nerves to injury.

If susceptibility to injury be connected with size, then the connection should reveal itself by an order of blocking in relation to the different velocities of conduction, since velocity is a linear function of the diameter of the fibers (Gasser and Erlanger, 1927). This order can be determined

by observation of the conducted action potential, because fibers of different velocities can then be identified on account of their temporal dispersion. If the small fibers be the first to be affected, the potential wave should be shortened from behind; if large fibers be first, the wave should be shortened from the front. Thus the effects of pressure and of cocaine may be predicted. Since they seem to act in a reverse order on the functions mediated by the nerve, they should also affect the action potential in a reciprocal manner, one shortening the front of the wave, the other the tail. Should this occur as predicted, then a comparison of the psycho-physiological observations with the behavior of the action potential should yield information as to the functions mediated by fibers of different sizes.

GENERAL METHOD. The nerve was stimulated at one end and the action potential, after amplification, was recorded monophasically from the opposite end by means of the cathode ray oscillograph. After the normal form of the action potential wave had been recorded, the block was applied to a short stretch of nerve between the lead and the stimulus. The shortness of the block was necessitated by the fact that conduction becomes slower before it is extinguished. To prevent too great confusion from the resulting relative shift of the constituent potentials in the combined wave, it was necessary for the conduction in the depressed area to be as short as practicable in comparison with the total distance of conduction.

In the interpretation of the experiments it should be borne in mind that the impulse is normal until it reaches the depressed area. There it drops in size or is blocked entirely. On emergence the impulse is again traveling in normal nerve and, according to the all-or-nothing law, the process in each active axon must be of normal size and velocity. What is being recorded is normal axon potentials. Any difference between the normal compound wave and one observed after the impulses have passed a depressed area must be due either to inactivity of fibers under the lead, owing to the fact that the impulses have been blocked, or to displacement of axon potentials due to slow conduction in the depressed area.

BLOCK BY COMPRESSION. The method used was a modification of that of Meek and Leaper (1911). Through a brass tube 12 mm. long having a 2.5 mm. bore was threaded a fairly thin-walled rubber tube, whose outside diameter fitted the bore of the brass tube. The ends of the rubber tube were everted over the ends of the brass tube and tied. Since the application of pressure led to protrusion of the rubber tube from its collar and to subsequent bursting, displacement was prevented by metal caps. These were perforated at the center for passage of the nerve. Pressure was applied from an oxygen cylinder through a side-arm of the brass tube, it being quickly, easily and accurately controlled by a reducing valve. To release the pressure after blocking, a needle-valve was inserted in the connecting tubing.

The mechanism of pressure block was not studied, only the results. However, by analogy with the effect of external pressure on thin-walled tubes, where the large tubes would collapse before the small ones, it was to be expected that the large fibers would be the first to be blocked; and such proved actually to be the case.

RESULTS. The effect of pressure was revealed most strikingly when about 25 pounds per square inch were applied rapidly through the cuff. The α wave then seemed to melt away from the fluorescent figure of the action potential on the screen of the tube. But for a careful analysis of the progress of the block it was necessary to build up the pressure more slowly. When this was done very concordant results were obtained, and they can best be presented in the form of an analysis of a single series. Eight of the fifteen records, made in this series during the course of the blocking, are reproduced in figure 1; and for further reference five of them have been redrawn in rectangular linear coördinates and superimposed (fig. 2).

When the pressure was applied the height of the α crest began to decrease; but simultaneously with this the form of the whole wave began to change, showing that the majority of the fibers in the nerve were already affected. When curve 3 was taken, the β crest and the notches on either side were already definitely delayed. This delay increased throughout the period of compression, and it occurred to a relatively greater degree in all the earlier parts of the action potentials. Between records 1 and 6 the α crest was delayed about 0.4 σ, while β was delayed 0.2 σ, and γ hardly at all. By the seventh record so many α fibers had been blocked, that the crest of the wave had no relation to the peak of the histological distribution curve of the α group; in fact, an approximate correction had to be made to locate the real α crest in record 6.

The course of the β crest could be followed more exactly. The height of the crest decreased slightly, rose again, then progressively fell away. According to the conditions of the experiment these changes can be explained only by a temporal redistribution of the constituent potentials or by blocking. While it is possible that some β fibers may have been blocked out of turn, a comparison of the areas of the waves shows that the initial decrease in height was due mainly to temporal dispersion. The subsequent rise in height was due to a delay of the faster fibers in the compressed area, so that they appeared beyond the block in the same position as smaller, less affected fibers. Later, with the blocking of the β fibers, the whole wave decreased in height just as the α wave had previously undergone the same change.

The γ wave was progressively delayed, but this delay did not become marked until the β wave was rapidly decreasing. Even in the late records the γ wave was essentially intact; but encroachments of the more delayed

faster fibers had filled up the notch between it and β, and had probably raised its crest.

In the late records it is seen that, beginning at the time of the start of the normal action potential, there is a long low potential lasting up to the start of the β wave. The most probable explanation of this is that a few fibers of very varied sizes had a more sheltered position with respect to the compression. Their number was small however, and the majority of the fibers followed the rule.

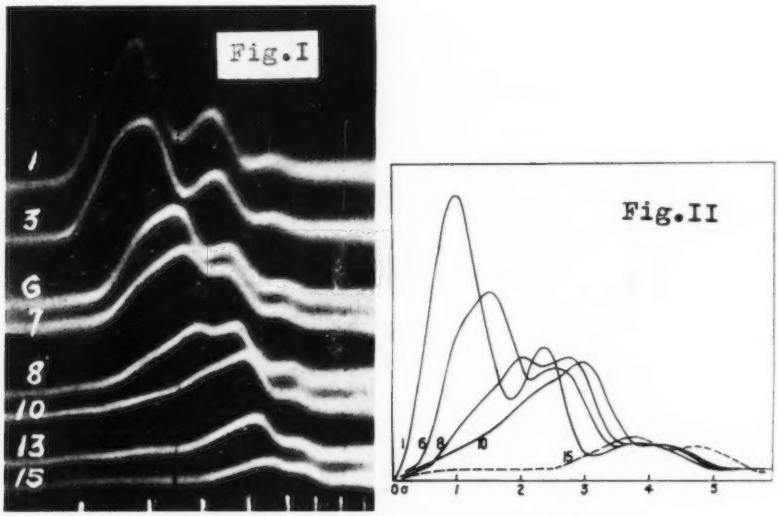

Fig. 1. Action potentials of a bullfrog nerve (10/28/26). 1 mf. 5000 ω. Conduction 7.8 cm. Time in sigma in all figures, *1*, 12:54 normal; 12:55 compression started with 10 pounds per square inch. *3*, 1:02; 1:09 pressure increased to 25 pounds per square inch. *6*, 1:10; *7*, 1:10:30; *8*, 1:11:15; *10*, 1:12:30; *13*, 1:14:45; *15*, 1:34.

Fig. 2. Records 1, 6, 8, 10 and 15 of figure 1, redrawn in rectangular linear coördinates and superimposed at their origins.

BLOCK BY COCAINE. For the application of the cocaine solution the nerve was threaded through two perforations in the side of a small vulcanite cylinder. When the cylinder was filled, the surface film at the openings was sufficient to maintain a column of liquid high enough to cover the nerve. Taking into consideration the amount of spread of solution along the nerve, the length actually in contact with the drug could not have been over 1 cm. The experiments on frog nerves were performed at room temperature, and those on mammalian nerves at 37° unless otherwise noted.

The results obtained with cocaine were not as uniform as was the case for

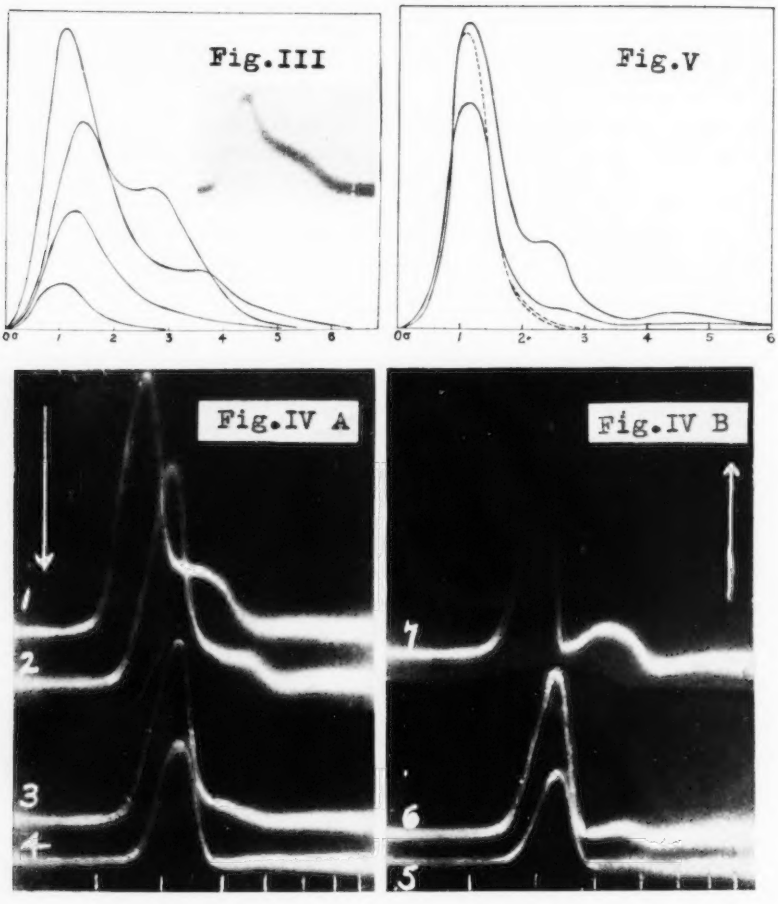

Fig. 3. Four stages of the compound action potential of a dog's saphenous nerve undergoing a block in 1/1000 cocaine HCl (10/9/26). Conduction 9 cm. The records were taken 4, 13, 17 and 21 minutes after the application of the drug. The largest wave differs from normal in having a deeper notch between the β and γ waves (first and second waves in saphenous). The form of the normal wave is seen in the reproduction of the actual record in the inset.

Fig. 4. Dilute cocaine on the sciatic nerve of the bullfrog (10/14/26). 1 mf. 5000 ω. Conduction 7.8 cm.

A. Period of block. *1*, normal, 10:23. At 10:28 1/10,000 cocaine HCl; at 11:58, 1/2000 cocaine HCl. *2*, 12:37; *3*, 1:20; *4*, 2:03.

B. Period of recovery beginning at 3:00. *5*, 3:23; *6*, 3:50; *7*, 5:00.

Fig. 5. Dilute cocaine on the sciatic nerve of the bullfrog (10/15/26). Conduction 8.2 cm. Records redrawn in rectangular linear coördinates. ———— whole action potential. ---------- α group only. The first parts of these are coincident with the whole action potential.

pressure. The amount of differential action was variable, and in some frog nerves was insignificant or zero. However, a differential action was visible in every mammalian nerve studied; and wherever it occurred, in nerves of all species, it was the same in kind: small fibers were blocked before large ones.

The experiments were performed in two series. The first series depended upon the fact that the conducted action potential occurs in waves, the method being to note the time at which each of these waves disappears. The first three waves α, β, and γ are very close together and represent the potentials of fibers larger than 5 μ. Following the γ wave and well separated from it is a fourth wave which was described by Erlanger (1927) in the saphenous nerve of the dog and labeled δ. This wave also occurs in other nerves and is best revealed in records made with four panel (100,000 fold) amplification.

For the separation of the α and β waves, the branches of the crural nerve promised to afford a favorable preparation, as Erlanger had observed that the muscle-branch receives only the first or α wave, while the saphenous branch receives fibers whose fastest component corresponds to the β wave of a mixed nerve. Accordingly, the preparation was arranged so that the undivided portion passed through the cocaine chamber in such a way that the proximal end could be stimulated electrically, and each of the two branches could rapidly be connected in turn to the amplifier.

In two of three preparations from the dog the waves in the saphenous branch disappeared before any great involvement of the muscle-branches. In the third preparation such a differentiation was absent, although there was good differentiation as to the time of disappearance of the waves within the saphenous nerve itself. This lack of uniformity was very possibly due to the existence of an uncontrolled variable in the connective tissue sheath of the nerve, as the sheath is a very important factor affecting the rate of the action of drugs applied perineurally. The branches of the crural nerve still have their own sheaths in the undivided portion of the nerve and therefore any variation in their thickness or holes at the points of cut branches would produce a serious complication. The sheaths on the two branches are about the same in thickness; but there are many divisions of the muscle branch at this level, while there are none of importance in the saphenous. This would place the muscle branch at a relative disadvantage.

In the saphenous nerve the δ wave always disappeared before the $\alpha\beta\gamma$ complex; and it was also seen to behave similarly in the tibial nerve of the cat and in the sciatic nerve of the frog.

The second series of experiments was limited to a closer analysis of the changes in the $\alpha\beta\gamma$ complex. In general, γ was more affected than β, and β more than α; but during the blocking of the slower waves the faster

waves were undergoing alterations in form. It was therefore necessary to make a special study, to determine whether or not some fibers in the faster waves were blocked before all the fibers in the slower ones.

In figure 3 are recorded the effects of cocaine on the portion of the $\alpha\beta\gamma$ complex found in the saphenous nerve, that is on the β and γ fibers. The experiment was performed at 25.5°C. Four records were selected from the series, enlarged optically, redrawn in linear coördinates, and then plotted so that they all start at the same point; although actually, when the last record was made, the fastest fibers took 0.6 σ longer than was normally necessary to traverse the 90 mm. distance of conduction. This meant that the fastest fibers had a mean velocity in the cocainized stretch of about 13.3 m.p.s., a value 30 per cent of normal. Block is anteceded by a great retardation in velocity.

A mere glance at the figure shows that the γ wave was blocked before the β wave, but that at the time of block the β wave was much reduced in height. This decrease in height can be explained in part by the greater temporal dispersion of the constituent fibers. But is it entirely so explained? To obtain an answer to this question some reconstructions were made of the action potential which would be predicted by the histological constitution of the nerve. The method for this procedure has been previously published (Gasser and Erlanger, 1927); and the data contained in figure 15 of that paper were employed. After a preliminary reconstruction, made for the conditions obtaining in a normal wave, had provided a control theoretical curve in satisfactory correspondence with the one actually recorded, reconstructions were made of the action potentials in partially blocked nerves, on the assumption that the velocity in fibers of each size would be the same fraction of normal as occurs in the fastest fibers. These reconstructions made it quite apparent that the fall in the height of the β wave could not possibly be accounted for on the basis of spread, either on the assumption selected or on any one employing a set of velocities which would permit the crests of the waves to fall in their proper positions.

Some of the large fibers might possibly be blocked ahead of small ones because of a position in the nerve trunk more accessible to the poison. They might, for instance, lie at the concentrated end of a diffusion gradient; but, if that be so, the effect should become minimal when very dilute solutions are employed to produce the block. Accordingly, an experiment was done on the sciatic nerve of the bullfrog, in which the block was started with 1/10,000 cocaine-HCl and finished with 1/2000. Records selected from this experiment are reproduced in figure 4 so that the abscissae correspond. The delay in the fastest fibers is therefore evident. The secondary waves decrease progressively in height, but in spite of the slow action of the poison the α wave also decreases in height as they disappear;

and it does not elongate sufficiently to explain the decrease. As the last record was taken 3½ hours after the start of the cocaine action, opportunity was almost surely given for an even distribution of the drug throughout the perineural spaces. Therefore, the blocking of some of the fast fibers before the slower ones cannot be explained as due to a diffusion gradient.

The recovery series for this experiment is also included in figure 4. As is usually the case the late waves are the last to recover; but in this particular experiment, part of the changes in form between the initial and final records must be due to changes independent of cocainization, as the total period of observation of the nerve was 6½ hours.

As a further test of the order of fiber disappearance, 1/2000 cocaine-HCl was applied to another bullfrog sciatic nerve and records made not only of the whole action potential but also of the α group alone, the latter being obtained by the use of an appropriately submaximal stimulus (Erlanger, Gasser and Bishop, 1924; Gasser and Erlanger, 1927). The drug acted very slowly and a few β fibers were still conducting after three hours. The records taken for figure 5 were made after a period of action of the drug lasting 2½ hours; they are redrawn in rectangular linear coördinates. The delay of the fastest fibers in passing the cocaine cell was small in this experiment, but the amount present has been eliminated in the figure so as to start all the curves at the origin.

The area of any part of the curve represents the area of the fibers in the nerve trunk which was responsible for that part. Therefore, with the aid of a planimeter, the areas of both the whole waves and of their corresponding α waves were measured. In the curve for the normal nerve the α wave constituted 58 per cent of the total, a value in good agreement with the percentage area of the α fibers in the nerve (vide Gasser and Erlanger, 1927, fig. 10). In the cocainized nerve the area rose to 83 per cent of the total, not, however, before the blocking of some fibers within the wave, as the wave-area was only 90 per cent of normal. In the meantime the whole action potential had fallen to 63 per cent of normal, which means that it contained the potentials of fibers other than α fibers amounting to 5 per cent of the original area.

The sum of the experiments shows that in general small fibers are blocked before large ones, but that the blocking is not effected with any precision. Fibers of all sizes are found to be unable to conduct as long as smaller ones. Thus, while fiber size is a determining factor in nerve susceptibility to poisons, it is not sufficiently differentiating to cause the fibers to drop out on a strictly size-basis. It may be for this reason that the psycho-physiological findings are not in better accord.

Discussion. The foregoing experiments show that, in so far as there is a differential action of cocaine on the fibers of a nerve trunk, it is the small fibers which are most affected. This might be due to the fact that their

thinner myelin sheaths would permit easier access to their axial protoplasm, but it is not necessary to make use of the myelin sheaths in formulating a sufficient explanation. If we assume that cocaine acts by chemical combination with the protoplasm, then, since the surface per unit volume increases directly as the diameter decreases, the smaller the fiber the greater the accessibility. At first sight, such an hypothesis suggests that when the nerve is washed the same factor would also cause the small fibers to be the first to recover. But this is definitely not the case. Recovery begins in the large fibers and proceeds in an order the reverse of that of the blocking, thereby corresponding with what has long been known with respect to the return of function. However, further consideration of the accessibility hypothesis suggests that a possible consequence would be that the protoplasm of the small fibers combines with the poison not only to the point of block but to a point far beyond this. The effect is progressive; block is preceded by varying degrees of slower conduction and it may be followed by a greater disorganization of function. Thus a longer period of washing would be necessary either to remove the excess drug or to allow more time for reorganization.

Such a simple mechanism as has just been described should cause the fibers to be blocked systematically on a size-basis; and since this does not rigidly hold the problem can be considered to be only partly solved. Some other as yet undetermined factor must be operating.

However, the relationship of the ease of blocking to the size of the fibers is sufficiently precise to explain the origin of the notion that sensory nerves are more easily blocked than motor. Small sensory fibers have been compared with large motor fibers; and, had it so happened that it would have been equally easy to compare vasomotor and proprioceptive fibers, the reverse notion could easily have arisen. In fact Dixon (1905) has described cardio-inhibition as disappearing more easily, on cocainization of the vagus nerve, than the reflexes modifying respiration. A motor function has disappeared before a sensory one, which probably means that it is mediated by smaller fibers.

Location of the various sensory fibers in a nerve trunk. Since the observations on the relative intensities of the action of pressure on the conduction of the sensations of cold and warmth cannot be made to fit with the action of cocaine, these two senses must be considered collectively as temperature. With this simplification, the two sets of data, derived from pressure and cocaine experiments, indicate that the senses in a nerve trunk are located, with respect to the fibers of various sizes, in the order, contact, temperature, pain. Use can be made of this order, provided the ends of the series can be located. Unfortunately this can only be done by inference. The most probable location of the upper end is in the β group, because this is the fastest wave in a cutaneous sensory nerve. The

lower end is more difficult to locate; but Ranson and Billingsley's observation (1916) that pain is mediated through the lateral division of the dorsal root, which is made up only of unmyelinated and small myelinated fibers, gives an indication as to its position. The other senses must fall in the intermediate fiber groups.

SUMMARY

An important factor, determining the relative susceptibility of the constituent fibers in a nerve trunk to a pressure and to cocaine, is their size. Pressure exerts its greatest effect upon the large fibers, cocaine upon the small ones. These facts offer an explanation of the order in which functions have been observed to be depressed, and they provide an aid to the location of these functions among the fiber groups.

BIBLIOGRAPHY

BAGLIONI AND PILOTTI. 1909. Zentralbl. f. Physiol., xxiii, 869.
BIER. 1899. Deutsch. Zeitschr. f. Chir., li, 361.
DIXON. 1905. Journ. Physiol., xxxii, 87.
DUCCHESI. 1901. Pflüger's Arch., lxxxiii, 38.
EFRON. 1885. Pflüger's Arch., xxxvi, 467.
ERLANGER. 1927. This Journal, lxxxii, 644.
ERLANGER, GASSER AND BISHOP. 1924. This Journal, lxx, 642.
ERLANGER, GASSER AND BISHOP. 1927. Ibid., lxxxi, 473.
ERLANGER, BISHOP AND GASSER. 1926. Ibid., lxxviii, 562.
FABRITIUS AND BERMANN. 1913. Pflüger's Arch., cli, 125.
GASSER AND ERLANGER. 1927. This Journal, lxxx, 522.
GOLDSCHEIDER. 1886. Pflüger's Arch., xxxix, 115.
GOLDSCHEIDER AND HAHN. 1924. Pflüger's Arch., ccvi, 308.
HERZEN. 1885. Pflüger's Arch., xxxviii, 93.
LAPICQUE AND LEGENDRE. 1913. Compt. Rend. Acad. Sci., clvii, 1163.
1922. Journ. de Physiol. et de Path. Gén., xxi, 163.
LAPICQUE, GASSER AND DESOILLE. 1925. Compt. Rend. Soc. de Biol., xcii, 9.
LUEDERITZ. 1881. Zeitschr. f. Klin. Med., ii, 97.
MEEK AND LEAPER. 1911. This Journal, xxvii, 308.
PONZO. 1908. Pflüger's Arch., xiv, 384.
RANSON AND BILLINGSLEY. 1916. This Journal, xl, 571.
SCHILDER. 1913. Zeitschr. f. d. Ges. Neur. u. Psych., xiv, 604.
THÖLE. 1912. Neurol. Zentralbl., xxxi, 610.
ZEDERBAUM. 1883. Arch. f. Anat. u. Physiol., 1883, 161.

SUBMERGENCE AND POSTURAL APNEA IN THE MUSKRAT

(FIBER ZIBETHICUS L.)

THEODORE KOPPÁNYI AND MARION S. DOOLEY[1]

From the Pharmacological Laboratory of Syracuse University

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It is generally recognized that diving animals show persistent inhibition of respiration while under water. In ducks it was shown by Paul Bert (1870) that submergence for as long as 16 minutes may not kill the bird. Huxley (1913) discovered in the duck an entirely new type of apnea which may be elicited by straightening the neck and dorsiflecting the head upon the neck. This reflex she called "postural apnea." Koppányi and Kleitman (1927) confirmed her results and observed the behavior of the heart during postural apneic pauses of several minutes' duration. Koppányi and Dooley further studied the postural apnea of the duck, especially the causes of cardiac slowing and blood pressure elevation which accompany postural apnea.

Huxley described the postural apnea as a mechanism to aid the diving bird in resisting the aspiration of water when the head is submerged. Although Koppányi and Kleitman could not detect any position of the duck which is characteristic of postural apnea when they submerged the bird forcibly, it is, however, possible that in certain phases of spontaneous diving the duck would make use of this postural reflex. This assumption would gain considerable support if it could be shown that other diving animals besides the duck show similar submergence and postural apnea. This, indeed, seems to be the case.

Paton (1927) discovered the presence of postural apnea in two other diving birds: the swan (Cygnis olor) and the cormorant (Phalacrocorax carbo), and Koppányi and Dooley observed postural apnea in the muscovy duck (Cairina moschata). The purpose of this investigation was to determine the presence or absence of postural apnea in an aquatic mammal.

EXPERIMENTAL. We chose for our experiments an easily obtainable diving mammal: the muskrat (Fiber Zibethicus L.). The muskrats used for our experiments are excellent divers and were in good condition when we obtained them except for the fact that one of their hind limbs was slightly injured by the trap. For our experiments we had the muskrats

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tied to an animal board in a supine position. The head and neck of the animal projected beyond the edge of the table so they could be easily dorsiflexed. All manipulations on the animals were performed under chlorbutanol anesthesia. In order to obtain records of the blood pressure and perhaps of the heart rate, we took regular blood pressure tracings inserting a three-way cannula into the right common carotid artery. The base line in all our tracings represented 0 mm. Hg pressure. The respiratory rate was recorded by means of a pneumograph tied around the body of the animal and connected with a tambour.

A. Submergence apnea. The normal respiration of the muskrat in the specimens we worked with was uniform and no periodicity such as Koppányi and Kleitman saw in certain ducks, or superimposed respiration as Swindle (1926) saw in certain muskrats, was observed.

When the muskrats were on their way to recovery from the anesthesia, and the reflexes were already present, the nostrils were slightly dipped under water, or, by means of a pipette, a stream of water was allowed to flow over the nostrils. At times not only the nostrils but the entire head of the animal was submerged under water. Whenever the animal's nostrils were brought in contact with water, we could uniformly observe an almost immediate cessation of respiration (fig. 1). During the period of submergence apnea the muskrats would execute general struggling movements from time to time. These movements transmitted by the pneumograph to the recording tambour could be misinterpreted as respirations (fig. 2). The inhibition of respiration may be seen even after removing the animal's head or nostrils from the water. This may be due to the fact that the mucous membrane of the nostrils is still wet. The blood pressure rose during submergence apnea (fig. 2). Unfortunately, the heart rate could not be detected on the blood pressure tracings, but by means of a stethoscope it could be discovered that it fell a few seconds after the beginning of submergence apnea.

B. Postural apnea. The postural reflexes of the muskrats were tested when the animals were on the point of recovery from the anesthesia. In our endeavor to elicit postural apnea we employed the same method which has been so successfully used in the duck. We gently grasped the animal's upper jaw (it must be remembered that the animal was in a supine position) and pulled on the neck so that it became straight and slightly stretched. Another method also often employed in the muskrats was that of dorsiflecting the head upon the neck, vertex of the head directed downward. To our surprise both of these manipulations resulted either in an appreciable slowing, or in a complete cessation of respiration. Very much as in the duck the respiration becomes exaggerated after releasing the animal's head and neck from the apnea position. This is very probably due to the accumulated CO_2 in the blood which stimulated the respiratory center

(fig. 3). If the chlorbutanol anesthesia is somewhat deeper, or is reinforced by morphine sulphate, the recovery of respiration following postural apnea is slow (fig. 4).

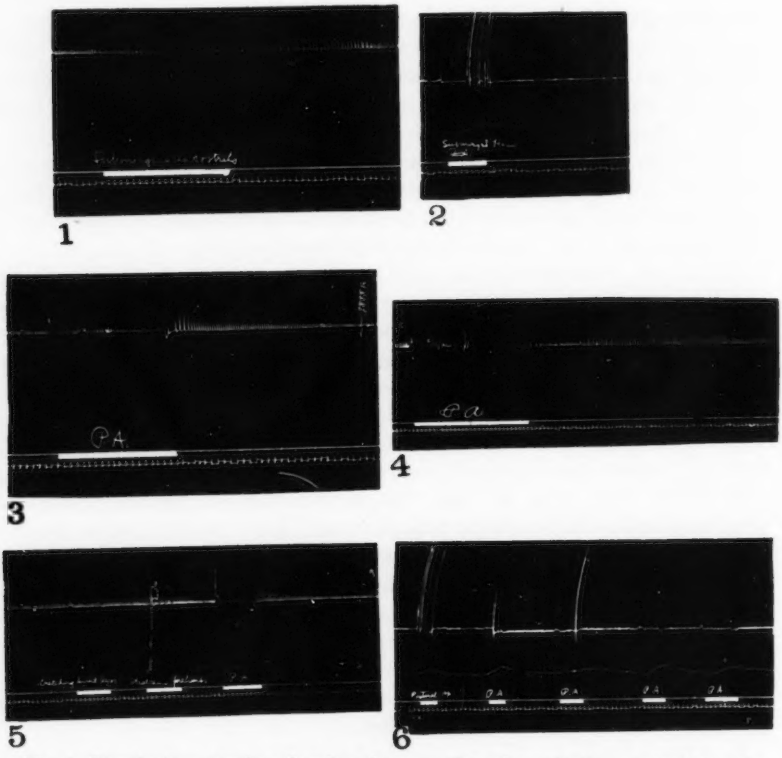

Fig. 1. Respiratory tracing showing the cessation of respiration caused by submergence of nostrils. Note slow recovery from apnea. Time 5 seconds.

Fig. 2. Simultaneous record of respiration (upper tracing) and blood pressure (lower tracing) showing the effect of submergence of the head on respiration and blood pressure. Time 5 seconds.

Fig. 3. Respiratory record showing postural apnea in the muskrat. Time 5 seconds.

Fig. 4. Respiratory record of the same animal more deeply anesthetized. Note the slow recovery from postural apnea. Time 5 seconds.

Fig. 5. Respiratory recortl of the muskrat showing the absence of effects of stretching the extremities on respiration. It also shows postural apnea. Time 5 seconds.

Fig. 6. Simultaneous record of respiration (upper tracing) and blood pressure (lower tracing) showing the effects of postural apnea on blood pressure. Time 5 seconds.

Since it was reported that in guinea pigs stretching of the extremities produces temporary cessation of respiration, we felt that we must check our postural apnea findings against the alleged effects of stretching. Although we could not see any appreciable slowing of respiration or apnea due to stretching of the limbs in the guinea pig we felt that we ought to perform a number of such control experiments even in the muskrat. As figure 5 shows, the stretching of the hind legs or fore limbs of the muskrat produced no change in respiration, whereas stretching of the neck produced apnea.

The blood pressure rose during postural apnea (fig. 6). The heart rate fell during postural as well as during submergence apnea.

Discussion. It was to be expected that the muskrats would show submergence apnea, but that they also show postural apnea is interesting from more than one point of view. First, it is evident that a mammal can show postural apnea, so that the peculiar respiratory apparatus of the bird is not necessary for the maintenance of postural apnea. Second, it is interesting that the respiratory, vasomotor and the cardio-inhibitory centers should react to stimuli in the muskrat as they react in the duck. It should be emphasized that since postural apnea occurs in the muskrat, and possibly in other diving mammals, there must be a genuine relationship between diving and postural apnea. Postural apnea is associated with diving and not with phylogenetically related taxonomic groups.

SUMMARY

1. The muskrat shows submergence apnea upon wetting of the nostrils.

2. Straightening of the neck or dorsiflexion of head upon neck produces postural apnea in the muskrat.

3. Both submergence and postural apneae are accompanied by elevation of the blood pressure and slowing of the heart.

BIBLIOGRAPHY

Bert, P. 1870. Physiologie de la Respiration. Paris.
Huxley, F. M. 1913. Quart. Journ. Exper. Physiol., vi, 183.
Koppányi, T. and M. S. Dooley. 1928. This Journal, lxxxv, 311.
Koppányi, T. and N. Kleitman. 1927. This Journal, lxxxii, 672.
Paton, N. D. 1927. Proc. Royal Soc. Edinburgh, xlvii, 283.
Swindle, P. F. 1926. This Journal, lxxix, 188.

PHYSIOLOGICAL FACTORS INVOLVED IN THE ELECTRICAL RESISTANCE OF THE SKIN

CURT P. RICHTER

From the Psychobiological Laboratory, Johns Hopkins Medical School

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It has been shown, in previous papers from this laboratory, that mental conditions are clearly reflected in the electrical conductivity of the body (Richter, 1926, 1928, 1929; Syz and Kinder, 1928). The experiments reported in the present paper are concerned with the physiological basis of this relationship.

The phenomenon is manifested in two ways: first, in a short transitory decrease in resistance which accompanies almost every emotional response; and second, in prolonged variations in resistance level occurring in different types of subjects, both normal and psychopathic, and in the same subject at different stages of mental activity and in varying moods. The brief, transitory change produced in the skin resistance by unpleasant stimuli such as pin pricks, loud sounds, etc., was first described by Féré in 1888. Since then, through the work of Veraguth (1908), this change has become known as the "Psychogalvanic reflex," and has been used to study the emotional responses of psychopathic patients. While engaged in an investigation of the physiological processes underlying these short reflex responses, we became impressed with the necessity of studying the basic conductivity levels upon which they are superimposed.

Until very recently little attention was given to the basic levels and few facts were known regarding them. A significant contribution was made by Vigouroux in 1879, however, when he found the electrical resistance very low in hyperthyroid patients and high in those regarded as hysterical. In partial corroboration of his data, we have obtained extremely low resistance readings in patients whose condition had been diagnosed as hyperthyroidism (5,000–20,000 ohms as compared to the normal average of 200,000–400,000 ohms). Our results with hysterical subjects have not been consistent, but we have found the resistance maintained at a very high level of 1,000,000 ohms or more throughout the psychotic period in patients in catatonic stupors. Similar chronic variations occur on a smaller scale, moreover, in normal subjects so that the resistance is low in tense individuals and relatively high in those free from tenseness. During sleep, in agreement with Waller (1917–19), and Farmer and Chambers (1924–25), we have noted a large increase in resistance which is roughly proportional

to the depth of the sleep (Richter, 1926). The question naturally arises as to the underlying physiological mechanism controlling the resistance of the body. How are changes in conductivity brought about in different subjects and in the same subject in different mental states?

Previous investigations have revealed two facts which are of prime importance for further study of this problem: *1.* We have found that the resistance offered by the body to the passage of a small constant current is localized practically completely in the skin, since a minute puncture made through the skin with a needle decreases the resistance from any level, however high, to zero (Richter, 1926). *2.* The skin resistance not only undergoes quantitative changes in the same individual, but shows qualitative variations as well. After preliminary observations of the conductivity of the skin on many regions of the body, we limited our studies largely to the skin on the palms and backs of the hands, and found that the resistance of the dorsal areas is normally about four times higher than that of the palmar surfaces. In eleven normal subjects, on whom hundreds of daily readings were taken, the average back-back resistance was 290,310 ohms and the palm-palm 77,100 ohms (Richter, 1928). Moreover, the resistance of the skin on the backs of the hands varies independently of that of the palms. The dorsal conductivity may decrease sharply while the palmar readings are increasing, as happens during sleep; and conversely, the palm-palm record may decrease while the back-back is increasing, as occurs very frequently in catatonic patients. The dorsal resistance shows the greater fluctuations from day to day, the palmar usually remaining constant except during periods of marked mental and physical disturbance. Lack of knowledge regarding these qualitative differences in skin resistance has undoubtedly given rise to the numerous conflicting results reported by various workers in this field.

With these facts as a basis an attempt has been made in the present investigation to determine first, in what parts of the skin,—sweat-glands, capillaries, epithelial cells or cornified layer,—the resistance is localized, and second, to what extent the resistance is controlled by nerve impulses and local conditions. How are the changes in conductivity brought about, and how are they related to other body functions, especially to the heat-regulating mechanism? Although the investigation has been under way for six years, these problems are still not completely solved.

EXPERIMENTAL METHODS. In the experiments reported below measurements were made of the resistance opposing the passage of an imperceptible constant galvanic current through the body. The electrodes consisted of bell-shaped pieces of pure zinc $1\frac{1}{4}$ inch in diameter with brass rods 2 inches long, screwed into the top, to serve as handles. The flat, smoothly polished surface of the zinc was covered with a quarter-inch layer of paste made of kaolin and saturated zinc sulphate solution. This

mixture served to establish contact between the zinc discs and the surface of the skin. Such electrodes are thoroughly satisfactory for work of the nature under discussion since they are non-polarizable, are quickly prepared and adjusted, make an intimate moist contact with the skin without injuring or irritating it, and allow considerable movement of the hands without changing the resistance. A further description of the electrodes and of the stands used to hold them in place is given in a previous paper (Richter, 1927).

The resistance was measured according to the following procedure by means of an Einthoven string galvanometer (Hindle model), in common use in electro-cardiographic stations. Before each reading the string was standardized without the patient in circuit, so that an electromotive force of 1 m.v. produced a deflection of 1 cm. on the scale. At this stage the only resistance in the circuit was that of the string. When the patient was introduced, however, the added resistance offered greater opposition to the current and consequently diminished the magnitude of the deflection, since the magnitude is roughly proportional to the strength of current and inversely proportional to the resistance. Then the electromotive force was increased until the same current was flowing as before, and the string was again deflected 1 cm. on the scale. The larger the resistance of the patient, the greater was the E. M. F. required to produce the original 1 cm. deflection. With these data the resistance of the patient in circuit could be calculated.

If, S = Resistance of string
R = Resistance of patient
N = Number of millivolts necessary to deflect the string 1 cm. with the patient in circuit,

Then, according to the formula,

$$\frac{\text{Electro-Motive-Force in volts}}{\text{Resistance in ohms}} = \text{Current in amperes}$$

without the patient in circuit,

$$\frac{1 \text{ m. v.}}{S} = C, \text{ the current flowing when the string is deflected 1 cm.}$$

and with the patient in circuit,

$$\frac{N}{R+S} = C, \text{ the current flowing when the string is deflected 1 cm.}$$

By equating,

$$\frac{1}{S} = \frac{N}{R+S}$$

$$R + S = N \times S$$

$$R = N \times S - S$$

$$R = S\,(N - 1)$$

We are well aware that this method of calculation is not perfectly accurate. The differences in resistance encountered in the various conditions

studied are so great, however, that they overshadow completely the unavoidable inaccuracy involved.

That resistance measured with a small constant current is *apparent* rather than *true* resistance, has been amply demonstrated by Gildemeister (1915) and his students, as well as by Ebbecke (1921) and others. Apparent resistance is always very much higher than true resistance because of polarization phenomena. It is well known that a current passing through living tissue gives rise to an opposing current, so that the resistance offered by the tissue appears much higher than it actually is. True resistance can be measured only with an alternating current of frequency sufficiently high to eliminate the polarization effects. It is very low, usually not higher than one thousand ohms, while apparent resistance, measured with a constant current, varies from a few thousand to several million ohms. Apparent resistance biologically is by far the more important phenomenon because it is so directly dependent on polarization and semipermeability, two of the chief characteristics of living cells and tissues, as established by Ebbecke's investigations of the "local galvanic reaction."

In all of the following experiments the resistance of the skin was measured separately on the palms and backs of the hands.

For this purpose the four electrodes, one attached to the palm and one to the back of each hand, were connected with the galvanometer by means of a switch-board wired with three double switches, so that a current could be passed through the body in six different ways, giving six resistance readings as follows:

(1) From the back to the palm of the right hand = R_1
(2) From the back to the palm of the left hand = R_2
(3) From the back of the right to the back of the left = R_3
(4) From the palm of the right to the palm of the left = R_4
(5) From the back of the right to the palm of the left = R_5
(6) From the palm of the right to the back of the left = R_6

From these six readngs, represented as R_1, R_2, R_3, R_4, R_5, and R_6 respectively, it is possible to calculate separately the skin resistance of the palm and back of each hand.

If, a = resistance of the right back,
b = resistance of the right palm,
c = resistance of the left palm,
d = resistance of the left back;

then,

$$R_1 = a + b$$
$$R_2 = c + d$$
$$R_3 = a + d$$
$$R_4 = b + c$$
$$R_5 = a + c$$
$$R_6 = b + d$$

From these six equations the values a, b, c, and d can be calculated algebraically. Moreover, they provide a means of checking every record, since

$$R_1 + R_2 = (a + b) + (c + d) = T,$$
$$R_3 + R_4 = (a + d) + (b + c) = T,$$
$$\text{and } R_5 + R_6 = (a + c) + (b + d) = T.$$

If the values for T obtained in each equation do not coincide within a small percentage of error, all six readings are repeated.

RESULTS. 1. *Physiological factors controlling the skin resistance of the palms of the hands.* A. *Sweat-glands.* Because of the great qualitative variations in skin resistance mentioned above, and because of the anatomical differences known to exist between the skin on the palmar surface of the hands and that on the dorsum, we have studied separately the physiological factors underlying the conductivity phenomena of these two regions. By what factors is the skin resistance of the palms of the hands controlled? The anatomical difference is expressed chiefly in relative porosity or distribution of sweat glands. It seemed logical, therefore, to begin our investigation with a study of the rôle played by these glands. Accordingly the skin resistance was measured when they were very active, and when they were completely paralyzed or inhibited.

Extreme activity of the sweat-glands, as judged by the actual appearance of moisture on the surface of the skin, could be produced most readily, we found, by the hot-air bath. A very satisfactory apparatus for this experiment consisted of a chamber large enough to enclose the patient's entire body with the exception of his head. This chamber was heated by ten carbon lamps so distributed as to insure a uniform temperature. A record typical of those obtained from many subjects in such a bath is shown in figure 1 a. The skin resistance in ohms is given on the ordinates; the time of the experiment in ten-minute intervals on the abscissae. The curve represents the total resistance of the two palmar surfaces. At 10:50 a.m., before the heat was turned on, the resistance was 140,000 ohms; at the height of the sweating reaction, when moisture was visible on the palms, the resistance dropped below 25,000 ohms; and fifteen minutes after the bath was discontinued and the sweating had ceased, it rose again to 280,000 ohms.

Similar records were obtained when sweating was produced by pilocarpine given subcutaneously in doses of $\frac{1}{5}$ grain in the upper arm well away from the hand. In the experiment recorded in figure 1 b, the resistance was 135,000 ohms before the injection, and 30,000 ohms when the pilocarpine effect was at a maximum.

The sudden excessive secretion of sweat which usually follows exercise

or severe emotional excitement, and the sweating of hyperthyroid patients, are also associated with a low resistance. Records compiled in eight cases

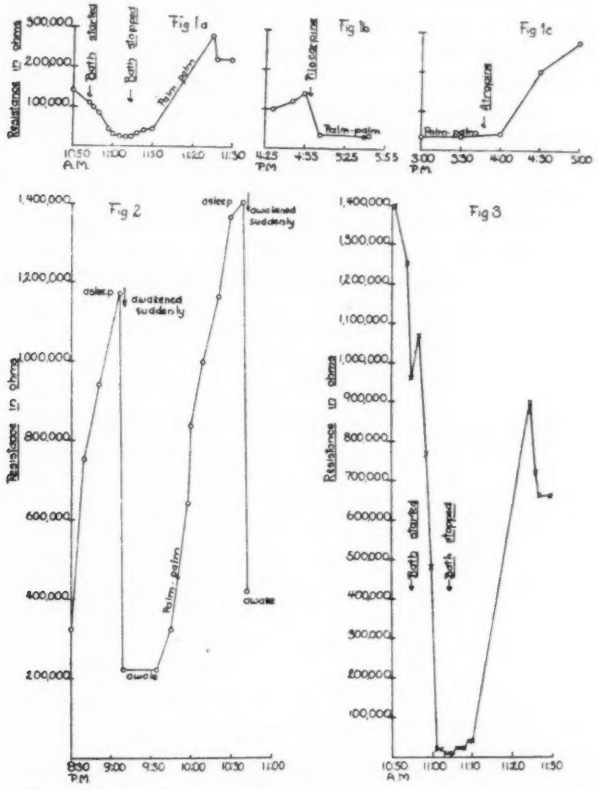

Fig. 1 a. Graph showing the effect of a hot-air bath on the skin resistance of the palms of the hands. The ordinates represent the resistance in ohms; the abscissae, the time. The arrows indicate when the bath was started and stopped.

Fig. 1 b. Graph showing the effect produced on the palmar resistance by hypodermic injections of pilocarpine.

Fig. 1 c. Graph showing the effect produced on the palmar resistance by hypodermic injections of atropine.

Fig. 2. Graph from a spider monkey, showing the large increase in palmar resistance occurring with sleep and the sharp decrease following sudden awakening.

Fig. 3. Graph showing the effect of a hot-air bath on the skin resistance of the backs of the hands.

of hyperthyroidism are summarized in table 1 in the column headed palm-palm resistance. The skin resistance of the palms averaged only 17,000

ohms in contrast to an average of 77,100 ohms obtained from a group of normal subjects (Richter, 1928).

All of these experiments concerned with the over-stimulation of the sweat glands were repeated many times on many different subjects with practically the same result. Invariably hyperactivity of the glands was associated with a low resistance of the palmar skin of the hands.

Conversely when the activity of the sweat glands was reduced through inhibition or paralysis, the resistance showed a great increase above the normal level. In forty subjects inhibition of the sweat glands by hypodermic injections of atropine ($\frac{1}{50}$ grain) in the upper arm invariably produced an increase in palmar resistance. In some the increase was very great, in others it was slight but still definite. A typical record is presented in figure 1 c. Before the injection the resistance was 25,000 ohms,

TABLE 1

Hyperthyroid patients

	PALM-PALM RESISTANCE	BACK-BACK RESISTANCE	BASAL METABOLISM
S. M.	15,000	10,000	+77
M. R.	13,000	13,000	+61
N. Q.	13,000	13,000	+54
R. T.	10,000	10,000	+64
R. B.	26,000	26,000	
A. W.	15,000	10,000	+78
P. I.	26,000	26,000	
M. M.	18,000	18,000	+80
Average	17,000	15,800	
Normal (11 cases)	77,130	298,330	

and an hour and a half afterwards it had risen to 280,000 ohms. It is interesting to note further that when this experiment was repeated several times on an individual with ichthyosis (congenital absence of sweat glands) no increase whatever occurred.

Similar results were obtained from monkeys when the sweat glands were paralyzed by transection of all nerves to the foot. The sciatic and femoral nerves were severed on one side in seven Macacus rhesus monkeys at a point between the hip and the knee, so that all innervation of the foot was abolished. In every case the palmar resistance rose to a high level immediately after the operation and was maintained at a high average for several months until the records were discontinued. The results of these experiments will be published in detail in another paper.

The relationship between the sweat glands and palm-palm resistance is

further illuminated by changes which occur in the resistance curve during sleep. According to the clinical point of view generally accepted, the sweat glands continue to function during sleep, whereas the other secretory glands, salivary, lacrymal, etc., are inhibited. On the basis of our observations, however, the sweat glands also appear to be inactive. Perhaps the discrepancy arises from the fact that the clinician, in observing the profuse moisture which often collects on the bodies of his patients during sleep, has not noticed on what parts it actually appears. We have found that usually the palmar surface of the hand with its numerous sweat glands becomes very dry during sleep, while excessive moisture may appear on other regions of the body, particularly on the trunk where sweat glands are scarce.

In a long series of investigations this dryness of the palms during sleep was accompanied consistently by a large increase in the palmar skin resistance. Records obtained from human subjects have already been published (Richter, 1926). In one instance the palm-palm resistance rose from 70,000 ohms before the patient went to sleep, to 1,450,000 ohms six hours later just before he was awakened. Figure 2 shows a similar record taken on a spider monkey. Small sheet electrodes were attached to his feet and he was put to bed in a baby's crib, where he was accustomed to sleep for six hours or more without waking. Before he fell asleep his resistance was 330,000 ohms. Within thirty minutes afterwards it rose to 1,160,000 ohms. Then when he was aroused suddenly it dropped at once to 220,000 ohms. Later, when he fell asleep again, the resistance rose to 1,400,000 ohms, and sudden awakening again produced an instantaneous decrease to 410,000 ohms. These results were confirmed many times in other monkeys.

It is important to note that in all of the above experiments, relative moistness of the skin is significant merely as a criterion of sweat gland activity. The resistance is not influenced to any appreciable extent by the actual presence of sweat either on the surface of the skin or in the tubules of the glands. The definitely graded changes of conductivity associated with sleep, for instance, occur in the absence of any visible or tactually perceptible moisture. It is difficult to see how differences in the amount of sweat in the tubules could account for such wide variations. If the resistance depended on the column of moisture in the tubules we should expect to find a very low resistance when the column is intact, and a very high resistance when it is broken, but no graded differences. We have found further that when the palms are sweating profusely and the resistance is low, careful cleaning and drying of the hands and the reattachment of fresh electrodes do not raise the resistance. It remains at the previous low level.

All of the above results may be resolved into the fact that the resistance

of the skin on the palms of the hands is controlled to a great extent by the activity of the sweat glands. When these glands are very active the resistance is low; when they are inhibited or paralyzed, the resistance is high. It remains to be seen to what extent, if any, the palmar resistance is dependent on other components of the skin, particularly the capillaries.

B. *Capillaries.* This phase of the problem has been investigated from several different angles. In the earliest experiments an attempt was made to determine whether the resistance changed with the marked blanching of the skin produced by subcutaneous injection of adrenalin. In forty different subjects a dose of 1 cc. of 1:1000 suprarenalin was injected subcutaneously in the upper arm. In almost every subject a slight increase in palmar resistance occurred, but there were marked individual differences in the magnitude of this increase. In view of the fact that adrenalin may have an inhibitory effect on the sweat glands as well as a constrictor effect on the capillaries, it is difficult to say whether this small increase should be attributed to the glands or the capillaries. In any case the increase was very slight in comparison to that produced by atropine, nerve section, or natural sleep.

An effort was made, also, to determine whether dilatation of the capillaries is accompanied by either an increase or a decrease in resistance. A pressure cuff left on the arm for as long as thirty minutes caused considerable engorgement of the capillaries, whether only the veins, or both the veins and the arteries were occluded. This was evident from the reddened appearance of the skin and was confirmed by actual observation of the capillaries with a microscope. A record from the palm of the other hand was taken as a control to make certain that the changes were not due to emotional stimulation. The experiment was performed on twelve different subjects, and in most instances the dilatation caused no significant change in conductivity. The few individuals in whom the resistance increased very perceptibly showed a comparable increase on the control hand, so that no special significance could be attached to this result. In two patients the resistance decreased sharply when the pressure cuff was released, but rose rapidly again to the original level.

Wells (1927) has found that vaso-constriction is associated with a decrease in skin resistance, and vaso-dilatation with an increase. It is very difficult to compare his results with our own, however, because he used liquid electrodes and the conductivity of relatively porous and non-porous areas could not be calculated separately. It will be noted that in our pressure cuff experiments, the palmar resistance showed a decrease if any change at all when the capillaries were dilated.

C. *Epithelial cells.* The question now arises as to whether the epithelial cells of the skin have anything to do with palmar conductivity. It is difficult to study the rôle played by them by any direct method, because

there are no simple criteria for estimating their activity. Ebbecke (1921) has recently described changes in skin resistance which arise in the epithelial cells. He has found that stimulation of the skin, whether mechanical, chemical, galvanic or thermal, produces a decrease in resistance limited strictly to the area stimulated, and he calls this phenomenon the "local galvanic reaction." He has been unable to produce it on the palms of the hands, although it is set up very easily on other parts of the body. We have repeated his experiments many times and have found likewise that the reaction cannot be elicited from the palmar areas. In view of very striking changes produced in this way on the backs of the hands and the absence of any effect on the palms we have concluded that the epithelial cells have no significant part in controlling the palmar skin resistance.

D. *Cornified cells.* Various other observations suggest that the palmar resistance is independent also of the cornified cells of the skin. We have found in studying a large number of subjects that the readings may vary widely from one individual to another, but bear no relation to the state of the skin whether it be tough and calloused or delicate and thin. Moreover, it is clear from the rapid changes that may occur in the palm-palm resistance, and its sensitive adjustment to various stimuli, that it could not possibly be controlled by dead cells. The statement that resistance is dependent on cornified cells has been made by those who have taken only an isolated record on each of their subjects and have consequently never observed how actively the individual resistance may change.

E. *Nervous control of the palmar skin resistance.* The nerve section experiments described above have shown definitely that the skin resistance of the palms of the hands is subject to nervous control, since transection of all nerves to either one fore-foot or one hind-foot in monkeys produces a great increase in the palmar resistance on the injured side simultaneously with the drying of the skin. This observation is borne out by our atropine and sleep experiments. It will be recalled that atropine, which is known to have a peripheral paralytic effect, produces a rapid increase in the palm-palm resistance. Natural sleep is accompanied by a similar increase in skin resistance, the resistance mounting higher as the sleep becomes deeper; and even more significant is the sharp decrease produced instantaneously by sudden awakening (see fig. 2). Such a phenomenon indicates that some nervous mechanism must be in control. But what is this mechanism? What part of the nervous system is involved?

Since the resistance changes so consistently with variations in the activity of the sweat glands, and since these glands are known definitely to be innervated by the sympathetic nervous system, we would expect the resistance to be subject to sympathetic control. We have been able to confirm this hypothesis by a series of experiments in which the sympathetic fibers to the foot were severed and the somatic fibers were left intact.

Sympathectomy alone produced as large an increase as the transection of the entire nerve; and subsequent transection of the remaining somatic fibers produced no further change. The only difference in results between sympathectomy and complete nerve section was that the former was followed by a temporary increase of a few months' duration, whereas the latter caused a permanent rise.

A further effort was made to determine to what extent, if any, the palmar resistance is modified by the reputed parasympathetic innervation of the sweat-glands, since it is a current view that the sympathetic and parasympathetic impulses oppose one another in controlling any of the vegetative organs. Our data are very meager but possibly worth mentioning at this point. We measured the skin resistance of several monkeys in which only the sympathetic innervation of the feet remained, all other impulses having been eliminated by section of the anterior and posterior roots within the cord. On the basis of the high conductivity readings obtained when the sympathetic fibers were severed, we expected very low records when they were left in complete control. And actually in all three monkeys, the palmar resistance remained almost at a zero level after the spinal roots were cut.

The fact that the skin resistance is high during sleep and low during the waking state might also be regarded as evidence in favor of this view since, according to Hess (1925) and others, sleep is a condition in which the parasympathetic nervous system predominates. It may be noted in this connection that the skin resistance does not reach a permanent waking level at once, but fluctuates widely for a few minutes after the sleeping subject has been aroused, so that the final level seems to be obtained through the balancing of two opposing forces.

To summarize, these experiments have shown by direct evidence that the palmar skin resistance is dependent to a great extent on the activity of the sweat-glands, and by indirect and less conclusive evidence that it is influenced very little, if at all, by the capillaries, epithelial cells, or cornified cells. Furthermore, the resistance is subject to nervous control from the sympathetic nervous system, and possibly from the parasympathetic as well. Impulses from the sympathetic component are known definitely to decrease the resistance; impulses from the parasympathetic may increase it.

2. *Physiological factors controlling the skin resistance of the backs of the hands.*

A. *Sweat-glands.* The hot-air bath experiment described above produced changes in the dorsal resistance curve very unlike those recorded from the palmar surfaces. Visible moisture could be produced on the dorsum of the hands only on rare occasions; although the skin became warm and moist to the touch, it did not show the profuse sweat found on

the palms. In spite of this fact, however, the back-back resistance decreased to a very low level during the bath, and afterwards, when the patients were cool and comfortable again, it rose almost to the original height. A record from a patient with an unusually high dorsal resistance is shown in figure 3. Before the bath was started the resistance was 1,400,000 ohms, when the heat was at a maximum it dropped to 20,000 ohms, and at the conclusion of the experiment it had risen again to 900,000 ohms.

Similarly, hypodermic injections of pilocarpine, which do not produce visible sweat on the backs of the hands, are often followed by a marked decrease in the dorsal skin resistance.

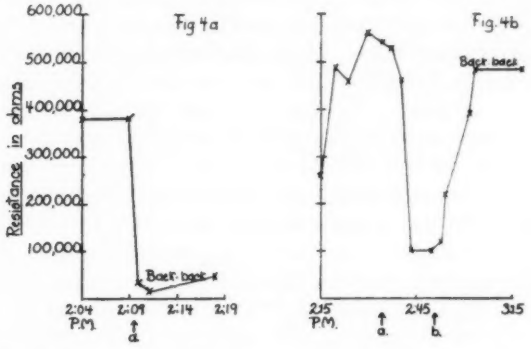

Fig. 4 a. Graph showing the effect of muscular effort on back-back resistance. At the point indicated by the arrow a heavy weight was fastened to the wrists while the arms were fully extended outward.

Fig. 4 b. Graph showing the effect produced on the skin resistance of the backs of the hands by changes in tenseness or strain. The first arrow indicates when the subject of the experiment was threatened with an electric shock; the second arrow, when he was told that he would receive no shock.

Mild exertion or exercise also causes a very rapid decrease in the dorsal resistance without changing the appearance of the skin in any noticeable way. A record which shows the effect of holding the arms in a horizontal position outward with small weights attached, is presented in figure 4 a. When the arms were in a position of rest, without the weights, the back-back resistance was 290,000 ohms. After the weights were attached and the arms were held extended for a minute, the resistance dropped to 20,000 ohms, without the appearance of any moisture.

Finally, it was found that the tenseness associated with emotional excitement has a similar effect on the conductivity, as may be seen from the graph in figure 4 b. The subject of this experiment was a colored laboratory boy on whom no records of any kind had ever been taken. He was

somewhat apprehensive while the electrodes were being adjusted, but was obviously relieved to find that nothing happened to him after they were in place. Coincident with his change of attitude a marked increase occurred in the back-back resistance from an initial level of 260,000 ohms to 560,000 within twenty minutes. Then he was told that in five minutes he would receive a shock, and this threat was embellished with a detailed description of the burns and blisters that might be produced by the current. As the boy became more and more frightened and nervous, the resistance decreased from 560,000 ohms to 100,000 within fifteen minutes, and it remained at this level as long as the painful stimulus was expected. When he was told, after ten minutes, that the shock could not be given on that day because the instrument was not working properly, he relaxed at once, and the back-back resistance rose in five minutes to a high level. No moisture was visible on his hands at any time during the record.

All of these experiments indicate that wide fluctuations may occur in the dorsal resistance, apparently independently of the activity of the sweat glands. Converse experiments in which the resistance changes were recorded when the sweat glands were inhibited, likewise demonstrated a complete independence. Thus, when atropine was injected hypodermically into the upper arm in forty subjects, the dorsal resistance was entirely unaffected and showed no tendency toward the increase found on the palms. Essentially the same result was obtained in the nerve section experiments in the monkeys. After all nerves to the foot had been severed the dorsal resistance remained unchanged, whereas the palmar rose, often far above 1,000,000 ohms. After death the dorsal resistance was unmodified despite the great increase which occurred in the palmar record. And finally, during sleep the back-back curve often showed a decrease. Here, however, the results were not consistent, since in some individuals the dorsal record increased with the palmar. The direction which the change took seemed to be governed largely by the nature of the sleep: when it was quiet and relaxed the dorsal resistance rose, and when it was disturbed and strained the resistance decreased.

The evidence presented in these experiments seems to indicate that, although the dorsal resistance varies with external heat and factors which have to do with heat-regulation, it is apparently independent of the activity of the sweat glands. It is well-known that the dorsum also contains sweat glands, although in less abundance than the palmar areas. Their effect, whatever it is, must be masked, therefore, by that of some other component of the skin.

B. *Capillaries.* Vaso-constriction was found to have no definite effect on the resistance of the backs of the hands. None of the forty subjects who were given adrenalin (1 cc. of 1:1000 solution) showed any dorsal conductivity changes either during the period when the skin was blanched or at any time thereafter.

Vaso-dilatation was equally ineffective. The marked engorgement of the capillaries produced by the occlusion of the veins alone or of the veins and arteries together with a pressure cuff, or that following transection of the nerves to the leg, caused no change in the resistance curve. According to the findings of Wells (1927), we would expect this dilatation to be accompanied by a large increase in resistance.

After the failure of these methods to show any relationship between the capillaries and dorsal conductivity, a new line of approach was followed. An attempt was made to discover whether any correlation existed between the magnitude of the skin resistance and the number of capillaries open in the skin. Records were taken on a group of subjects showing a wide range of individual variations, and each subject was studied for a period of several days to check any variations that might occur from day to day.

The number of capillaries open each day in a standard area of skin was counted by Dr. E. B. Carrier according to a method she had used previously in Krogh's laboratory in Copenhagen. The standard area consisted of the field enclosed within a rectangle formed by crossed hairs placed in the ocular of the microscope. In order to make counting simpler the rectangular field was divided into twelve squares; the capillaries were counted in one square at a time, and the numbers in each were added together. To make certain that the rectangular field enclosed the same area from day to day, one corner of the rectangle was placed over a spot made on the skin with india ink. The skin was covered with cedar oil and illuminated by a powerful, cooled light from an arc lamp, impinging on the surface at an angle of approximately forty-five degrees. In this way it was found that, although a low resistance and a large number of open capillaries occasionally occurred together for two or three days, on the whole the two phenomena seemed to fluctuate independently of one another. Furthermore, when the capillaries were counted in several subjects especially selected either because of an extremely high or extremely low back-back resistance, nothing unusual was found.

We hoped then that a solution of the question might be gained by a study of skin resistance in certain pathological conditions of the circulatory apparatus. For this reason records were taken on eight patients suffering from edema of the nephritic type. In all eight patients the dorsal resistance was found to be unusually high, far above the normal average. Because knowledge is so scanty concerning the basis of the edema condition, the records contribute little to the present problem. However, the fact that these patients did show very high readings consistently is interesting in itself, even though its significance is not yet clear.

In two patients suffering from hypertension an extreme lability in skin resistance was observed. In both of these patients the slightest exertion, such as arising from bed or walking across the floor, produced a rapid

decrease in the back-back resistance, while rest in bed for only a short time caused the resistance to rise quickly to the original height (see fig. 5). These observations might be regarded as positive indication of a correlation between electrical resistance and the capillaries, or at least some part of the circulatory system. Further evidence suggests, however, that the conductivity phenomena in these individuals probably occur indirectly through the effect of the circulation on the epithelial cells.

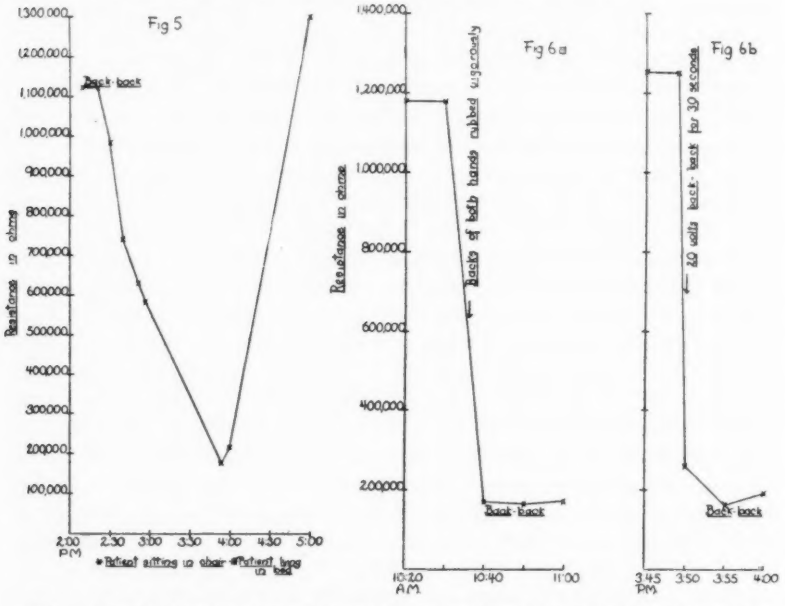

Fig. 5. Graph showing changes in resistance produced by activity and rest in a patient with hypertension.

Fig. 6 a. Graph showing the effect produced in the resistance of the skin on the back of the hand by vigorous rubbing with a Turkish towel for a few minutes.

Fig. 6 b. Graph showing the effect produced on the skin resistance of the backs of the hands by a galvanic current,—20 volts passed through the skin for 30 seconds.

C. *Epithelial cells.* The relation of the epithelial cells to the dorsal resistance was studied according to the methods used by Ebbecke in his investigations of the "local galvanic reaction," as described above. In keeping with his observations we obtained results on the backs of the hands very different from those obtained on the palms. Mechanical, chemical, thermal, and galvanic stimuli all produced a large decrease in dorsal resistance. From the graph in figure 6 a, for instance, we see that rubbing the backs of the hands for a few minutes with a turkish towel decreased the resistance from 1,180,000 ohms to 170,000.

When the skin was stimulated with a galvanic current we found, in agreement with the observations of Gaertner (1882), that its permeability was greatly increased. In the experiment recorded in figure 6 b, a current from a 20 volt battery, flowing from the back of the right hand to the back of the left for thirty seconds, caused the resistance to drop immediately from 2,060,000 ohms to 260,000. It must be noted, however, that there are large individual differences in the nature and magnitude of the responses set up by the current, and that in one subject the resistance may drop almost instantaneously, while in another the decrease may be slight and gradual, even when the current is applied for ten minutes or more. Currents with a potential varying from 5 to 20 volts were passed through the body without interruption during intervals varying from 2 to 60 seconds, but in no instance was the intensity or the duration of the current so great that it became painful to the subject, and no blisters were caused by the current. This fact is important in view of the results reported by Lewis and Zottermann (1927). They contend that the conductivity decrease produced by a galvanic current is due to a mechanical injury caused by the formation of gas bubbles under the skin. The results of the present experiment invalidate their statement.

Thermal stimulation has practically the same effect on back-back resistance. When the hand was held dorsal surface upwards directly beneath a 100 watt Jumbo lamp for five minutes, the dorsal resistance usually showed a marked decrease which persisted for fifteen minutes or more.

Chemical stimuli, such as ammonia and strong salt solution, produce similar results, but we have not studied them as extensively as we have the others.

It is evident that all agents which stimulate the skin produce a decrease in resistance. Conversely, Ebbecke has found that narcotizing agents such as chloroform vapor, which reduce the activity of the epithelial cells, cause an increase in resistance. In accordance with his results we believe that these changes in resistance are due directly to the changes in the epithelial cells, and that the layer of these cells functions like a membrane, becoming more permeable with stimulation and less permeable with inactivity or narcotization. The most conclusive evidence for this view is contained in the fact that a puncture made through the skin on the dorsal surface with a needle, however fine, reduces the resistance instantaneously from any height to zero.

D. *Cornified cells.* It has been asserted by Ebbecke and others that the high skin resistance often encountered immediately after the electrodes are attached is due to the extreme impermeability of the cornified stratum of cells, and that the effect of these cells is eliminated only after they have been soaked by the liquid of the electrodes for fifteen minutes or more.

Ebbecke believes, in fact, that the resistance of the cornified layer actually obscures resistance changes in the epithelial layer, and that it is the lower level reached after the short preliminary period that is dependent solely on the epithelial cells. According to him the "local galvanic reaction" cannot be studied, therefore, until the initial high resistance of the cornified layer has been eliminated.

Our observations, made on many subjects under various conditions, do not bear out this view. In the first place we have found in agreement with Thouless (1925–26) that the resistance does not always decrease after the electrodes are attached. Sometimes it increases, depending largely on the attitude of the subject (see fig. 5 b), or, as in the case of the narcoleptics, on some specific neuro-pathological condition (Richter, 1929). The resistance may rise within a few minutes, shortly after the electrodes are attached, from practically zero to a million ohms or more, or it may fluctuate in a short interval from a preliminary high level to zero, and then back to the original height again. Obviously such changes could not arise from any alterations in the dead tissue, but must have their origin in active living cells,—as we believe, in the epithelial layer.

F. *Non-nervous control of the dorsal resistance.* The experiments described above have shown clearly that the resistance of the skin on the dorsum of the hands is not subject to nervous control, since none of the nerve transection operations in the monkeys produced any changes in the back-back resistance curve. Similarly, atropine, which paralyzes the nerve endings to the sweat glands, has no effect on the dorsal record; and sudden awakening from deep sleep, which causes an instantaneous decrease in palmar resistance, changes the dorsal curve very slightly, if at all. Usually the back-back resistance decreases slowly, and does not reach a low level until several hours after the subject has awakened.

The question now arises as to how the changes in the epithelial cells of the skin are brought about, if not through nerve impulses. We have seen that these changes may be produced externally by mechanical, thermal, galvanic, or chemical stimulation. It is very probable that they may be produced from within the body in a similar way. Thermal stimulation of the epithelial cells may occur, for instance, either through an increase in the temperature of the blood flowing through the skin, or, more directly, through an increase in the amount of heat given off locally by the muscles. In the same way the cells may be activated by chemical agents carried in the blood stream, by action currents given off by the muscles in a state of contraction, or by changes of pressure transmitted from the vascular loops. Densham and Wells (1927) believe that changes in the permeability of the epithelial layer are due to variations in pressure exerted against the cell-walls by the vascular loops when dilated and contracted.

In summary we would say that the dorsal resistance appears to be de-

pendent largely on the activity of the epithelial cells, and influenced very little, if at all, by the sweat-glands, capillaries, or the cornified cells. Furthermore, it is controlled not by nerve impulses, as is the palmar resistance, but by local influences, such as the temperature of the blood, chemical agents in the blood, galvanic currents from underlying muscles, etc.

REMARKS. It is obvious that all of the various facts detailed above must bear some definite relationship to the function of the body as a whole. Sufficient data are not available at present to warrant any complete formulation of such a relationship. With this ultimate aim in view, however, our experimental results may be correlated very simply with certain phases of the heat-regulating mechanism of the body, since the conductivity phenomena of the skin on both the palmar and dorsal surfaces of the hands can be interpreted as functions of the heat-regulating process.

It has been shown that the palmar resistance is dependent on the sweat glands and is controlled by nerve impulses arriving through the sympathetic nervous system. Moreover, it varies inversely with the body-temperature, since it is low when the body-temperature is high and increases as the temperature descends below its normal level. If these observations are sound, then certain facts concerning the normal function of the sweat glands become apparent. These facts have already been observed according to other methods by other workers in this field.

Loewy and Wechselmann (1911) believe that the sweat glands are not active under ordinary circumstances but function only under emergency conditions when there is actual danger that the body-temperature may rise above its normal level. Schwenkenbecher (1925) and Moog (1927) contend, on the contrary, that these glands are in a constant state of partial activity under ordinary conditions during the waking state. Our data support the latter view, since the average waking palmar skin resistance is far above the zero level found when the glands are maximally activated, but still well below the level of complete inactivity (Richter, 1928). The fact that the palmar resistance remains so constant from day to day, as well as at times when the individual is undergoing marked changes in muscular tension, suggests that under ordinary conditions the sweat glands contribute a relatively constant amount of moisture in the heat-regulating process, and that the varying demands for thermal control are met through some other medium.

Various investigators today (Keeton, 1924; Richardson, 1926; Eimer, 1927) believe that under ordinary conditions the insensible perspiration mechanism of the skin is largely responsible for the maintenance of normal body-temperature. It has been demonstrated recently that this mechanism depends not entirely on the sweat glands, as has formerly been supposed, but on epidermal cells as well. It will be recalled that the dorsal

resistance seems to depend almost entirely on the epithelial cells of the epidermis. We believe, therefore, that a close relationship may exist between insensible perspiration and dorsal skin resistance. Loewy and Wechselmann contend that the moisture evaporates from the skin in a purely physical way. We agree with Schwenkenbecher, on the other hand, that it is dependent on a physiological process,—on the changes in the permeability of the cell membrane. Our attempts to confirm this theory have thus far been unsuccessful because of technical difficulties. Assuming it to be true, however, we may conclude, on the basis of the large fluctuations which occur in the dorsal resistance with daily variations of mood and muscular tension, that ordinary demands for heat-regulation are met chiefly through the insensible perspiration from the epidermal cells.

One additional observation concerning the interrelationship of palmar and dorsal resistance and sweating and insensible perspiration may be mentioned. Eimer (1927) has demonstrated that a reciprocal relationship often exists between sweating and insensible perspiration and that when one is high the other is low and vice versa. We have observed a similar relationship between palmar and dorsal resistance. In catatonic patients, for instance, whose dorsal resistance is abnormally high the palm-palm record is very low; and conversely, when the palmar resistance is at a maximum, after atropine injection, nerve section, etc., the dorsal curve is usually very low.

SUMMARY

1. An attempt was made in the experiments reported above to establish a physiological foundation for numerous observations made on the electrical resistance of the body in various normal and pathological conditions, such as sleep, stupor, narcolepsy, etc.

2. From previous observations it was known that the resistance offered by the body to a direct constant current is localized in the skin. It had been observed, also, that qualitative as well as quantitative variations characterized conductivity phenomena, since the resistance of the porous skin on the palms of the hands changed quite independently of that of the relatively non-porous skin on the backs. For this reason these two areas of skin were studied separately and an effort was made to determine for both areas what components of the skin,—sweat glands, capillaries, epithelial cells, or cornified cells,—control the resistance.

3. It was found that the sweat glands control the palmar skin resistance and that the capillaries, epithelial cells, and cornified stratum play an insignificant rôle, if any at all. The resistance decreased greatly when the sweat glands were stimulated to hyperactivity, and increased when they were inhibited. These experiments showed further that the palmar resistance is controlled by nerve impulses.

4. The skin resistance of the dorsal surface of the hand was found to be dependent primarily on the epithelial cells, and very little, if at all, on the sweat glands, capillaries, or cornified cells. The conductivity of these areas of skin appears to be controlled by local conditions. The skin of the dorsum, in other words, may be regarded as a semi-permeable membrane the permeability of which increases when the skin is stimulated by mechanical, thermal, galvanic, or chemical stimuli, and decreases when the stimuli are removed.

5. Both the palmar and dorsal resistance changes can be brought into relationship with the heat-regulating mechanism of the body, the palmar with the sweat glands and sweating, and the dorsal with the epithelial cells and insensible perspiration. These changes are further correlated with the mental condition of the individual, varying with degrees of tenseness or strain, with sleep, and with all the other normal diurnal changes in mood and disposition.

BIBLIOGRAPHY

Densham, H. B. and H. M. Wells. 1927. Quart. Journ. Exper. Physiol., xviii, 175.

Ebbecke, U. 1921. Pflüger's Arch., cxc, 230.

Eimer, K. 1927. Arch. f. Exper. Path. u. Pharm., cxxv, 150.

Farmer, E. and E. G. Chambers. 1924–25. Brit. Journ. Psych. Gen. Sec., xv, 235.

Fèrè, C. H. 1888. C. R. Soc. d. Biol., 217.

Gaertner, G. 1882. Wien. Med. Jahrb., 519.

Gildemeister, M. 1915. Pflüger's Arch., clxii, 489.

Hess, W. R. 1925. Schweiz. Arch. f. Neurol. u. Psychiat., Neurol. u. Psychiat. Abhandlungen, Heft 2.

Keeton, R. W. 1924. This Journal, lxviii, 307.

Lewis, T. and Y. Zottermann. 1927. Journ. Physiol., lxii, 280.

Loewy, A. and W. Wechselmann. 1911. Virchow's Arch. f. path. Anat., ccvi, 79.

Moog, O. 1927. Zeitschr. f. d. ges. exper. Med., liv, 226.

Richardson, H. B. 1926. Journ. Biol. Chem., lxvii, 397.

Richter, C. P. 1926. Proc. Nat. Acad. of Sci., xii, 214.

1927. Brain, l, 216.

1928. Arch. Neurol. and Psychiat., xix, 488.

1929. Arch. Neurol. and Psychiat., xxi, 363.

Schwenkenbecher, A. 1925. Sitzungsb. d. ges. z. Beförder d. ges. Naturwissenschaften zu Marburg., Nr. 1.

Syz, H. C. and E. F. Kinder. 1928. Arch. Neurol. and Psychiat., xix, 1026.

Thouless, R. H. 1925–26. Brit. Journ. Psychol., Gen. Sec., xvi, 5.

Veraguth, O. 1908. Das psychogalvanische Reflexphänomen, Berlin.

Vigouroux, R. 1879. Compt. rend. de la Soc. de Biol., xxxi, 336.

Waller, A. D. 1917–19. Proc. Roy. Soc., B, xc, 214.

Wells, H. M. 1927. Quart. Journ. Exper. Physiol., xviii, 33.

THE EFFECT OF ULTRA-VIOLET RADIATION ON BLOOD FORMATION IN YOUNG PIGS

FRANK P. MATHEWS, L. P. DOYLE AND R. A. WHITING

From Purdue University, Agricultural Experiment Station, Lafayette, Indiana

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In a previously published work (1927) on anemia in pigs, we found that the incidence of the disease was much higher when the pigs were reared in a central hog house than it was when the animals were kept outside at least part of the time. Moreover, the incidence of anemia in pigs kept under outside conditions appeared to be influenced by the proportion of cloudy days: i.e., when the proportion of cloudy days was high, the incidence of anemia was relatively high; when the proportion of cloudy days was low, the incidence of anemia was relatively low. Since the foregoing work was published, further observations have confirmed the opinion that, during the seasons in which the pigs are ordinarily farrowed, there is something in outside conditions which tends to prevent anemia.

The high incidence of severe anemia under inside conditions suggests that the sun's rays may play an important part in normal blood formation in the young pig. There is a rather general belief that rays from the sun and from some artificial sources play an important part in hematopoiesis. However, a survey of the literature did not furnish much convincing experimental evidence either to support or to disprove this opinion. The controversial nature of the results obtained by various investigators who have attempted to determine the effects of radiations on blood formation is apparent from a recent review of the literature by Laurens (1928).

EXPERIMENTAL. Our previous observations on anemia in swine suggested that the young pig is admirably suited for studying the effect of irradiation on hematopoiesis. Consequently, four young pregnant sows were kept inside of a well-lighted hog house throughout the period of gestation and were irradiated by means of a mercury vapor quartz lamp. The building in which the sows and their pigs were housed during the experiment was well lighted by means of ordinary glass windows. The irradiation of the sows was begun approximately 59 days before the pigs were farrowed. The length of the irradiation period was gradually increased from 15 to 40 minutes. The lamp used was "The Uviarc Poultry Treater" manufactured by Cooper-Hewitt. It was suspended 28 inches above the backs of the animals during irradiation. The sows were irradiated daily, except Sunday. Two of them were irradiated until their pigs were 13 days

old, while the other two were irradiated until their pigs were 38 days old. White rats were fully protected against rickets by daily exposure to the rays of this lamp for 10 to 15 minutes.

The pigs farrowed by these sows were first irradiated when one day of age. At first, the period of irradiation was 15 minutes, but was gradually increased to 40 minutes. The pigs were irradiated daily except Sundays, until they were 38 days of age. The experiment ended in May, 1927.

Another lot of six sows was used to furnish pigs which served as controls in the experiment. These sows were kept inside the hog house throughout the period of gestation, and until the experiment ended, but were not irradiated. For these sows, the basal ration was supplemented with 3 per cent cod liver oil. The basal ration consisted of ground yellow corn, 61; meat scraps, 15; linseed oil meal, 3; yeast, 3; bran, 5; wheat middlings, 11; alfalfa meal, 3; and mineral supplement, 2. The composition of the mineral supplement was as follows: calcium carbonate, 39; steamed bone meal, 39; sodium chloride, 18.75; ferrous lactate, 3; and potassium iodide, 0.25.

When the pigs of the non-irradiated sows were one day of age, three of the litters were divided as nearly equally as possible, and one portion of each litter was put outside on a concrete floor twice daily, except Sundays, until the pigs were 36 days old. At first, the periods of outside exposure were 30 minutes, but were gradually increased to two hours. However, the total amount of outside exposure did not exceed 70 hours.

Red cell counts and hemoglobin determinations were made on the blood of each pig, beginning when the animals were one to three days old and continuing at intervals of 7 to 10 days until they were 35 days of age. The hemoglobin determinations were made according to Newcomber's (1919) method. A white blood cell count was made when the pigs were 35 days old. The blood was obtained by cutting off the ends of the pigs' tails with a sharp knife and allowing the blood to flow freely before taking the samples.

Results. Table 1 shows the red cell and hemoglobin content of the blood of the three groups of pigs at different ages. It is seen that at one to three days of age, the average red cell and hemoglobin content of the blood was slightly higher in the pigs farrowed by irradiated sows than it was in the pigs farrowed by non-irradiated sows. This difference, however, is probably too small to be regarded as significant. It is also seen that at first there was a decrease of red cells and hemoglobin in the blood of all three groups of pigs. The hemoglobin content continued to decrease in the irradiated as well as in the non-irradiated pigs which were kept inside. At 35 to 38 days of age there was no difference in the two groups of pigs as regards red cell and hemoglobin content of the blood. The hemoglobin content of the blood of both of these groups was considerably below that of the group which was exposed to direct sunlight.

Table 2 gives the results of the total white blood cell counts which were made when the pigs were 35 days of age. It is seen that there was quite a wide variation in the white cell content of the blood of individual pigs, but that the difference between the averages of the three groups was not very great.

There was a striking difference in the death rate in the two groups of pigs which were kept inside. Thirty per cent of the non-irradiated pigs died when they were from 4 to 38 days of age, while 6 per cent of the irradiated pigs died during the same age period. The death rate in the pigs given outside exposure was 12 per cent.

TABLE 1

Average red cell and hemoglobin content of the blood of three groups of pigs

	AGE							
	1 to 3 days		7 to 11 days		17 to 22 days		15 to 38 days	
	R.B.C.	Hb.	R.B.C.	Hb.	R.B.C.	Hb.	R.B.C.	Hb.
Outside	3.6	7.7	3.0	5.1	3.6	6.3	4.4	7.1
Inside, non-irradiated	3.8	7.7	3.1	5.3	2.9	4.9	3.7	4.6
Inside, irradiated	4.2	8.8	3.2	5.9	3.4	5.3	3.7	4.7

R. B. C. = Millions of red cells per cu. mm. of blood.

Hb. = Grams of hemoglobin per 100 cc. of blood.

The number of pigs in the various groups when 1 week of age was as follows: 15 outside; 45 inside; not irradiated; and 44 inside, irradiated.

TABLE 2

Number of leukocytes per cu. mm. of blood of the three groups of pigs, at 35 days of age

	MAXIMUM	MINIMUM	AVERAGE
Outside	32,000	9,000	20,600
Inside, non-irradiated	43,000	4,000	17,450
Inside, irradiated	50,000	9,000	15,700

Discussion. The failure of mercury vapor quartz lamp radiations to produce any appreciable increase in red blood cells or hemoglobin in pigs, is in general agreement with results reported by investigators who used other animals in attempts to determine the effects of ultraviolet irradiation on hematopoiesis. The fact that the death rate was much less in the pigs which were irradiated by means of the mercury vapor quartz lamp than it was in the pigs which were not irradiated, suggests that irradiation had a beneficial effect other than the stimulation of blood formation. It should be remembered that cod liver oil was added to the ration of the non-irradiated sows and pigs in order to supply the anti-rachitic factor.

The increased red cell and hemoglobin content of the blood of the pigs

which were given outside exposure is in agreement with the rather general belief that sunlight stimulates hematopoiesis. Although this opinion is doubtless correct, the literature does not contain an abundance of unequivocal experimental evidence that the sun's rays stimulate blood formation. The well-marked increase of hemoglobin and red cells which occurs in the blood of people on going to higher altitudes may apparently be satisfactorily explained on a basis other than the increased intensity of sunlight. The work of Sherman and Rosenquist (1900) and Seiller indicated that this increase of red cells and hemoglobin is due to the decreased atmospheric pressure or, more particularly, to the decreased pressure of oxygen. According to Koranzi, the increased red cell content of the blood which occurs at high altitudes, as well as that occurring in cardiac insufficiency, quickly returns to normal upon inhalation of oxygen. It seems likely, then, that this increased hematopoiesis, which is sometimes cited as evidence that sunlight stimulates blood formation, is merely a compensatory action on the part of the body to adapt itself to a decreased oxygen supply.

There is fairly general agreement that the treatment of tuberculous patients with the direct rays of the sun, and in some cases with the rays of a carbon arc lamp, is followed by an increase in the red cell and hemoglobin content of the blood. It is probable that this improved blood picture is the indirect result of an improved physical condition rather than a direct response to light rays.

Even after discounting any evidence which is questionable, there is still a very strong suggestion that certain kinds of radiations stimulate blood formation, particularly in the young. It seems likely that clear-cut experimental evidence on this question could be obtained by using an experimental animal which is well suited for such studies. The fact that young pigs develop a severe primary anemia when kept under inside conditions, suggests that these animals are eminently suited for studying the hematopoietic effect of irradiation.

SUMMARY

Irradiation by means of a mercury-vapor-quartz lamp did not increase the red cell or hemoglobin content of the blood of young pigs.

BIBLIOGRAPHY

Doyle, L. P., F. P. Mathews and R. A. Whiting. 1927. Bull. 313, Purdue Agric. Exp. Station.

Koranzi. Quoted by Berner, Strahlentherap., v, 342.

Laurens, H. 1928. Physiol. Reviews, viii, 1.

Newcomber, H. S. 1919. Journ. Biol. Chem., xxxvii, 465.

Seiller. Quoted by Berner.

Sherman and Rosenquist. 1900. Quoted by Berner.

EFFECT OF VARIOUS INHALED VAPORS ON RESPIRATION AND BLOOD PRESSURE IN ANESTHETIZED, UNANESTHETIZED, SLEEPING AND ANOSMIC SUBJECTS

WILLIAM F. ALLEN

From the Department of Anatomy of the University of Oregon Medical School, Portland

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The subjects of these experiments were normal individuals, an anosmic and selected hospital patients under light ether anesthesia. The normal subjects and the anosmic were students who had done some laboratory experimentation and were willing to coöperate in every way. With this type of subjects the psychic impulses of fear and surprise were very well eliminated and all other psychic phenomena were excluded in so far as possible. With the exception of the anosmic all of the subjects were fully up to the average in the functional activity of the olfactory and trigeminal systems. All of the tests on the non-anesthetized subjects were conducted during the summer months when they were comparatively free from infections of the upper respiratory tract.

Technical procedure. The various vapors were inhaled from cones held below the nostrils, the distance varying with the nature of the substance. All subjects, not under an anesthetic or asleep, were blindfolded and their ears were stopped with cotton. Continuous tracings were recorded during the time a subject was inhaling from a blank cone and from a second cone containing the odoriferous or irritating substance. An assistant manipulated the signal magnet by observing the time when the second cone was substituted for the blank cone and the time when the cone containing the test substance was withdrawn. If an inhalant proved too irritating the subject signaled by a movement of the thumb. After a test had been recorded the subject was asked to describe the sensation and if possible to name the vapor inhaled.

Thoracic-respiratory tracings were obtained after the manner described in the first report of a previous series. A glass tambour strapped over the carotid artery and connected with a Marey's tambour by tubing served to record the carotid pulse. The systolic blood pressure was taken with a sphygmomanometer from the forearm of the subject for a period of a minute. During the first thirty seconds several systolic readings were taken at the time a blank cone was being held below the nostrils of the subject who was blindfolded, and a like number of readings were taken during the

remaining thirty seconds in which the subject was inhaling the test substance.

This method obviously involves the personal equation of the individual recording the blood pressure. In every subject some of the readings were checked by two individuals. Doctor Baird and Mr. Straumfjord took simultaneous readings from the right and left arms of the anosmatic subject. Neither recorder knew the inhalant used and each wrote his readings independently of the other. Upon comparing these data and other data it would appear that any change of the systolic pressure, amounting to 2 mm. or more during the inhalation of a vapor is of significance, but anything less than 2 mm. is entirely within the limits of normal records independent of inhalants.

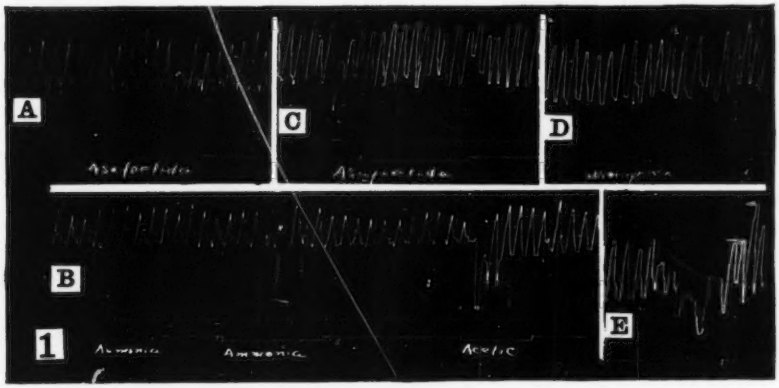

Fig. 1. Thoracic respiratory tracings, inspiration upstroke, time in 5 seconds; tracings A and B from normal subject A asleep, tracings C to E from normal subject A awake.

The writer desires at this time to express his thanks to Doctor Manville, Doctor Baird, Mr. Straumfjord and Mr. Schwichtenberg for taking these readings.

Respiratory tests on normal subject A. asleep and awake. The first part of this experiment is concerned with the effect or various vapors on the thoracic respiration of my 18 year old son when he was sound asleep. Numerous tracings taken while he was asleep demonstrate that respiration had taken place at the regular rate of a little less than 18 excursions a minute. There is, however, some irregularity in the amplitude of these excursions, in that occasionally one would be short. A comparison of the tracings obtained with oil of cloves, orange, rose, lavender, bergamot, butyric acid, asafetida (fig. 1 A), menthol, eucalyptus, camphor, xylol,

benzol, ether and chloroform with the preceding normal excursions reveals no more alteration of respiration than that which occurs during normal respiration. The same negative results were obtained on repeating the foregoing tests. As will be shown in the second part of these tests, when the subject was awake, the inhalation of asafetida produced a pronounced effect on respiration, so that this substance was inhaled frequently during the sleeping tests to determine whether the lad was actually asleep.

To the left in figure 1 B ammonia vapor of strong concentration was inhaled for 22 seconds without awakening the subject or altering his respiration. Fifteen seconds later ammonia was inhaled for the second time for a period of 25 seconds. This time (B, center) there is a marked depression of two inspirations, a deepening of the next two, a rapid return to normal upon removal of the inhalant and the boy was partially awakened. As a result of inhaling acetic acid (fig. 1 B, right) the first inspiration is stopped immediately and followed by several deep expirations. After this stimulation the return to normal includes several deep inspirations. This vapor woke up the subject completely.

During the second part of this experiment the above mentioned vapors were inhaled by subject A. fully awake. These tracings and others made two days later with the boy awake show that inhalation of the agreeable odors—oil of cloves and bergamot—produces a lowered inspiratory phase of the first few excursions without any change in their rate. Tracings from the disagreeable odors asafetida (fig. 1 C), butyric acid and fresh cat's urine reveal very irregular excursions during the interval of stimulation, some showing a deepened exhalation phase and others a considerably depressed inspiratory phase, their rate remaining unchanged. The xylol and alcohol graphs disclose a lengthened inspiratory stage and no change in the rate of excursion. Tracings obtained during the inhalation of wintergreen (fig. 1 D), camphor, menthol, ether, chloroform and pyridin demonstrate a tendency throughout stimulation toward both a shortening and lengthening of the inspiratory phase of the excursions, chiefly a depression. Some of these substances, ether, chloroform and benzol also produce considerable deepening of the expiratory phase of the excursions. Respiratory excursions in the records obtained at the time of inhalation of the very irritating vapors from oil of mustard, acetic acid and ammonia (fig. 1 E) always portray a marked shortening of the inspiratory phase, some increase in the expiratory phase and a reduction in rate of excursion or a complete arrest of respiration.

Respiratory tests in normal subjects C., S. and W. Of these three individuals, W. is the most sensitive and reacts strongest to the mild agreeable odors which are specific olfactory stimuli for rabbits. The respiratory excursions in the tracings obtained from C. and W. at the time of inhalating bergamot, oil of cloves (fig. 2 A) and lavender exhibit a considerably depressed

inspiratory phase and some increase in number; while the excursions from S. portray a lengthened inspiratory phase and a decrease in number, the actual rate of movement appears to be unchanged. The effect of the disagreeable odors—asafetida (fig. 2 B from W) and butyric acid in all three subjects is one which shortens the inspiratory phase and increases the number of excursions. Wintergreen produces a marked depression in C. and W., while in one set of tracings in S. it evokes a deepening of the inspirations and in a second set of tracings there is a marked depression of the first four or five inspirations and a deepening of the remainder. The excursions in the tracings for the mild irritants and odors—camphor, menthol, eucalyptus, peppermint, ether, chloroform, alcohol, xylol, benzol (fig. 2 C.

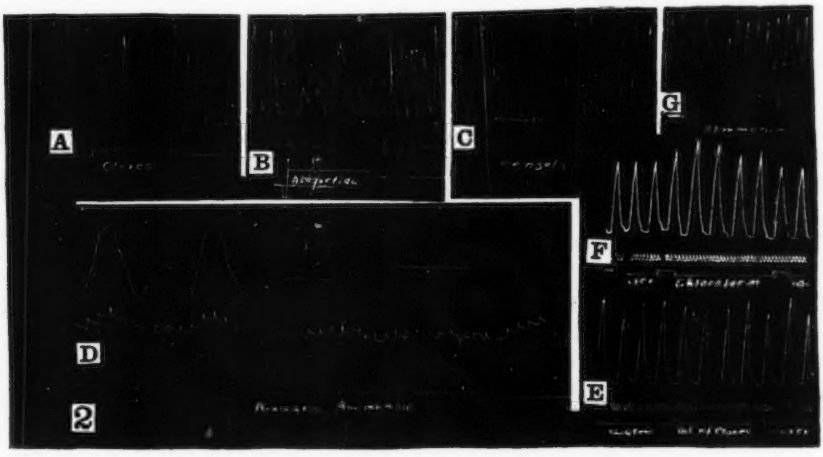

Fig. 2. Same as figure 1; tracings A to D from normal subject W, the tracing below the respiratory tracing in D is from the carotid artery; tracings E to G are from the anosmic.

from W.) and phenol reveal a considerably lowered inspiratory stage, frequently an increased expiratory stage and usually an increased number of excursions in C. and W.; while in S. the excursions are lengthened in both the inspiratory and expiratory stages. As a result of inhalation of strong concentrations of the very irritating vapors—oil of mustard, acetic acid and ammonia (fig. 2 D from W, upper tracing) there is a marked depression and slowing of the excursions or complete suppression of respiration in all three subjects.

In those records where considerable suppression of ventilation has taken place during the stimulation period, removal of the inhalant is followed by a considerable increase in ventilation. This may be produced by an acceleration or a deepening of the respiratory movements or both (fig. 1 E).

Some respiratory graphs were obtained from a small boy who was said to have a very acute sense of smell. The lad proved to be such an excitable chap that the large number of body movements recorded made these tracings worthless.

Respiratory tests in the anosmic subject H. This student has had no sense of smell for seven years as the result of a skull fracture caused by a falling telephone pole. A description of the fracture from x-ray plates by Drs. A. E., Paul and Eugene Rockey to the Oregon Accident Commission is as follows: "A comminuted fracture appears on the left side of the skull, consisting of two horizontal fractures in the temporal region which join a vertical fracture. The fracture lines extend into the vault of the pharynx and through the sphenoid and ethmoid sinuses." Bleeding was described from the nose and the left ear and there was a paralysis of the left rectus lateralis eye muscle. As a result of this accident the patient completely lost the sense of smell. The following tests, however, reveal that at least a branch of the trigeminal nerve to the nasal mucosa is functioning.

Tabulated data from two sets of tracings taken on different days demonstrate that inhalation of the agreeable and disagreeable odors—oil of cloves (fig. 2 E), orange, rose, lavender, bergamot, asafetida, butyric acid and fresh cat's urine produce no change in respiration. Xylol and wintergreen also gave negative results. The following mild irritants and odors—menthol, eucalyptus, camphor, peppermint, ether, chloroform (fig. 2 F) and benzol produce a considerable augmentation of the inspirations, and the amount of reduction in the rate of the excursions suggests that the actual rate of respiratory movement is unchanged. Weak concentrations of the strongly irritating substances, such as formalin, acetic acid and ammonia yield similar reactions to the mild irritants; while strong concentrations of the same substances (fig. 2 G, ammonia graph) cause the excursions to show a marked depression of their inspiratory phase and some increase in rate. If strong concentrations of mustard are to elicit any change in the respiration of this subject the cone must be held close to the nostrils. The effect which comes on slowly is one which increases the depth of both inspirations and expirations, together with some decrease in the rate of excursion.

After a respiratory tracing had been obtained the anosmic was asked to describe the effect of the substance. His answers show that none of the agreeable or disagreeable odors were perceived. Fresh cat's urine was not detected, but old decomposed cat's urine, possessing a trace of ammonia, was said to produce a different sensation than a strong concentration of ammonia did. Oil of wintergreen, camphor, menthol, eucalyptus, xylol and benzol were not detected, though some of these substances altered his respiration. Pyridin was described as a taste sensation coming from the back of the throat and tongue. Peppermint caused a cooling sensation in

the nose and ether a disagreeable burning sensation appearing quickly. On the other hand, chloroform elicited a pleasant sensation in the nose. Formalin, described as a tickling sensation in the nose, was a long time in appearing. A weak concentration of acetic acid or ammonia produced a burning sensation in the nose and throat, which would have resulted in a movement of the head if inhalation had been continued longer. Very singularly, this subject could inhale oil of mustard for a considerable length of time without any discomfort if the cone was held at 4 or 5 cm. from the nostrils. In fact, the change in respiration appeared before the sensation was detected.

Blood pressure tests. The subjects were the same as were used in the previous respiratory tests. It was found that the blood pressure of one subject engaged in manual out-door labor dropped 10 mm. after sitting quietly for an hour and from then on maintained a fairly constant level, so that all of the subjects were given an hour's rest before any readings were taken. The general technical procedure has been outlined in the introduction. As in the previous respiratory tests every attempt was made to exclude psychical stimuli from entering into these reactions. For example, it was found that the blood pressure from subject S. not only reacted very strongly to the first inhalation of butyric acid, but also showed a similar blood pressure change for every substance inhaled thereafter. The subject felt that he could still detect the butyric acid which was extremely repugnant to him. In view of this observation the agreeable odors were tested first, then the mild irritants, the disagreeable odors and finally the strongly irritating substances. As stated in the introduction only a change of 2 mm. or more in blood pressure during the inhalation of a vapor was considered of any significance.

A summary of various readings in the different subjects is shown in table 1.

Carotid pulse tests. A large number of graphs were obtained from the same subjects as were used for the respiratory and blood pressure tests, including the anosmic and my son asleep and awake. The same vapors were used for the inhalations.

When the pulse waves from these tracings were counted and their height measured before, during and after stimulation, the conclusion was reached that these data are of little comparative value and are therefore not included in this report. It is true that many graphs taken during the inhalation of benzol, ether, chloroform, formalin, ammonia, etc., show changes in the height and rate of the waves during the inhalation period. It is also true that the same vapors frequently do not alter the pulse in the same individual. Pulse tracings taken during the inhalation of the very irritating substances usually show abrupt changes in the height and depth of their waves which are the result of swallowing or other movements of the head and neck.

TABLE 1

VAPOR INHALED	EFFECT, SUBJECT A	EFFECT, SUBJECT C	EFFECT, SUBJECT S	EFFECT, SUBJECT W	EFFECT, ANOSMIC
Orange-lavender	No	No	No	No	No
Bergamot	No	No	No	No	No
Cloves				No	No
Peppermint	No	No	No	No	No
Wintergreen	6 mm. D	3 mm. R	4 mm. D	7 mm. R	No—2 mm. R
Menthol	4 mm. D	No (4)	2 mm. D (1)	3 mm. R	No
Eucalyptus		No (4)	3 mm. D	5 mm. R	No—2 mm. R
Camphor	4 mm. D (1)			5 mm. R	No
Ether	No—2 mm. D	5 mm. R	4 mm. D	9 mm. R (5)	2–4 mm. R
Chloroform	No	No	4 mm. D (2)	No	4 mm. R
Xylol	No	4 mm. D (3)	No	No	No
Asafetida	No—2 mm. D (2)	2 mm. D	No (2)	No	No
Butyric acid	No	No (4)	4 mm. D (2 and 3)		No
Pyridin	4 mm. R (2)	4–6 mm. D	4 mm. D (4)		4 mm. R
Formalin	4 mm. R (2)		15 mm. R (2)		5 mm. R
Ar. Sp. Ammonia	7 mm. R		4 mm. R.		2–4 mm. R
Acetic acid		5 mm. R (4)	4 mm. R (2)		5 mm. R
Oil of mustard	8 mm. R		14 mm. R		No—2 mm. R
Ammonia	Big. D (2)	8 mm. D (2)	20 mm. R (2)		10 mm. R (2)

In this table D and R represent drop and rise in blood pressure: (1) indicates a transitory drop in blood pressure, (2) movements of the head, (3) probably psychic effects, and (4) swallowing reflex or movements of the lips.

Several points of general interest were noted when the tracings were taken on my son while he was asleep. As was the case during the respiratory tests, inhalation of the agreeable, disagreeable and mildly irritating substances did not awaken the lad, but on the other hand, the first inhalation of ammonia awoke him immediately. It was observed in these tracings that his pulse became arrhythmical for some time after inhalation of several agreeable and disagreeable odors and xylol and benzol. This may be comparable to the so-called pseudo-vagal reaction frequently obtained in rabbits after fatigue from much inhalation or from several inhalations of benzol.

Some plethysmograms were taken from the forearm of the anosmic during the inhalation of several vapors, but independent movements of his arm produced so many irregularities that these records could not be used.

Respiratory tests on subjects under light ether anesthesia. Through the courtesy of Doctor Cliff, Director of Multnomah Hospital, the writer was permitted to make these inhalation tests on a number of surgical cases immediately after operation. The subjects selected were free from upper respiratory infections and were not likely to suffer shock from the operations. Each subject is referred to by number—1, a young woman (Bartholin cyst), 2, a young man (osteomyelitis of the femur), 3, a young man (osteomyelitis of the humerus), 4, a negro boy (skin graft on arm), 5, middle-aged man (bone graft) who was deeply under ether after the operation and showed signs of shock, 6, young woman (salpingectomy), 7, young woman (appendectomy).

Directly after the operation the patient was removed to a warm room, where thoracic respiratory tracings and systolic blood pressure readings were taken during the inhalation of oil of cloves, asafetida, wintergreen, xylol and ammonia. It was found that if a respiratory reaction is to be obtained from odors the subject must not be too deeply under the influence of ether or under what may be a combination of ether and natural sleep.

The respiratory graphs from all subjects obtained during the interval of inhalation of very strong concentrations of ammonia (fig. 3 A from 2) disclosed a complete suspension of respiration. Weaker concentrations produce a marked depression and slowing of the respiratory movements. Of the five subjects tested, wintergreen ordinarily produced the next strongest alteration of respiration, always causing a marked inhibition. The excursions in a graph from subject 2 (fig. 3 B) exhibit a shortening of the inspiratory and expiratory phases, the rate remaining unchanged. In subject 1 it evoked a slowing of the rate as well as a shortening of the excursions and in subject 4 respiration was brought to a standstill.

Xylol varied in its ability to depress respiration but its effect is almost nil when compared to its effect in rabbits. The xylol tracings from subjects 2, 5 and 6 are very similar to the asafetida tracing of figure 3 C. The

excursions portray only a slight depression which affects both the inspiratory and expiratory phases. The depressive influence is more marked in the tracings from subjects 1 and 4, while there was no change in respiration in subjects 3 and 7. The only suggestion of any slowing of respiration occurs in the tracings from subject 1.

Respiratory tracings obtained during the inhalation of the disagreeable odor asafetida reveal only a very slight depression of the excursions in subjects 2 and 7 (fig. 3 C from 2). In subjects 3 and 4 the depression is slightly more pronounced, taking place in the expiratory phase of the excur-

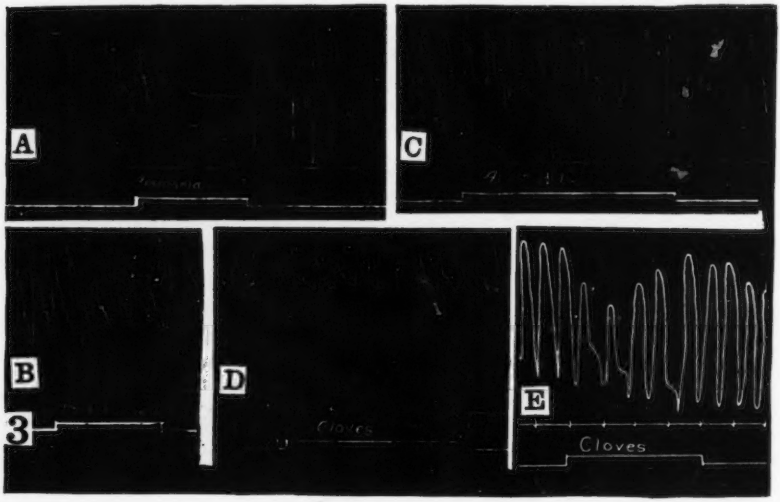

Fig. 3. Thoracic respiratory tracings from anesthetized subjects 2 and 1, inspiration upstroke, time in 5 seconds; A, ammonia tracing from 2; B, wintergreen from 2; C, asafetida from 2; D, oil of cloves from 2; E, photographic print of a tracing made from a non-contrasty oil of cloves graph from subject 1; the gradual drop throughout the tracing was caused by a small leak in the balloon.

sions in 3 and in the inspiratory phase in 4. In 4 the expirations are somewhat deepened. Respiration is unaltered by asafetida in subjects 5 and 6, the former was deeply under the anesthetic and the latter may have passed into a natural sleep when this tracing was taken. Unfortunately no test was made of asafetida in subject 1, whose respiration was always slowed as well as shortened by the other inhalants.

Oil of cloves, which was inactive in the anosmic and is a specific olfactory inhibitory stimulant in some rabbits, produced an inhibitory effect on respiration in all subjects but 5. The lack of response in this subject may be attributed to the depth of the anesthesia and shock. Figure 3 D, an oil

of cloves tracing from subject 2, shows a shortened inspiratory phase of the excursions with no change in their rate. The oil of cloves tracings from subjects 3, 4, 6 and 7 disclose about the same amount of depression of their excursions as is portrayed in figure 3 D. In subject 3 the depression is one which involves the expiratory phase of the excursions. Figure 3 E, which is a photographic print from a tracing of a non-contrasty oil of cloves graph from subject 1, shows a slowing of the respiratory rate in addition to the usual shortening of the excursions, apparently causing a reduction in ventilation proportionate to that obtained in rabbits with this substance.

Several systolic blood pressure readings were taken from the earlier anesthetized subjects before and during the period of inhalation. So many independent changes were observed in the pressure before the inhalations were given that these tests were discontinued.

COMMENTS. Previously reported experiments on rabbits have shown that the respiratory and vascular changes obtained during the inhalation of the odoriferous or irritating vapors are produced chiefly through stimulation of the olfactory and trigeminal nerve endings in the nasal mucosa.

Ponzo, Russi, and Malan have independently studied the effect of inhalations of several substances in man. I have not seen Ponzo's and Russi's papers, but Malan states that Ponzo advised his subjects as to the nature of the experiment and explained to them that they were to breathe deeply at a given signal and continue to do so until told to stop. His quoted results show that agreeable substances permit of deep inspirations, while disagreeable ones cause irregularities. Malan quotes Russi as concluding that agreeable substances produce prolongation of the inspiratory phase and disagreeable ones act on (lengthen?) the expiratory phase.

Malan used a very different procedure than Ponzo. His normal subjects, chiefly soldiers, were blindfolded and were not told what was to be done to them. Pneumograms and sphygmograms were taken during the inhalation of the essence of violets, scatol and acetic acid. This author found that each individual reacts in a different way to these substances, there being no constant relation to the stimulus employed. Violets produced acceleration of respiration in 15 per cent of the cases, inhibition in 5 per cent and no change in 80 per cent; scatol caused acceleration in 25 per cent of the cases, inhibition in 13 per cent and no change in 62 per cent; while acetic acid resulted in *acceleration* in 25 per cent of the cases, *inhibition* in 7 per cent and *no change* in 68 per cent. Concerning the pulse changes violets produced acceleration in 64 per cent of the cases, a slowing in 13 per cent and no change in 23 per cent; scatol evoked an acceleration in 53 per cent of the cases, a slowing in 26 per cent and no change in 21 per cent; acetic acid caused an acceleration in 49 per cent, a slowing in 9 per cent and no change in 42 per cent.

It is apparent from the foregoing inhalation-respiratory tests on unan-

esthetized normal subjects that the writer's results do not accord with the "laws" of Ponzo or of Russi, neither do they show the same irregularities or as many negative reactions as Malan portrays. It would seem to the writer that Malan's procedure would be especially adapted to produce psychic impulses from fear and surprise, which would invalidate his results as reflex effects. The writer always obtained a very definite inhibitory response from inhalation of the powerful irritants such as acetic acid and ammonia, strong concentrations usually producing a complete arrest of respiration. Two types of altered respiration result from inhalation of the mild irritants and the agreeable and disagreeable odors in normal unanesthetized subjects—1, a shallow type, consisting of shortened inspirations or a mixture of shortened inspirations and deeper expirations, usually accompanied by some increase (sometimes no change or a decrease) in the rate of the excursions; 2, a deep type, usually showing a decrease (sometimes no change) in the rate of the excursions. The first type should ordinarily contribute to a decreased ventilation, while the second type would probably cause little or no change. Ordinarily the same subject will react about the same for a given substance, but tracings from the same individual have shown type 2 reaction one time and type 1 or a combination of 1 or 2 another time.

In unanesthetized subjects blood pressure changes are less easily produced than in rabbits, and furthermore, they are not always a rise in pressure. No changes occurred in my subjects from inhalation of the agreeable odors and in some individuals from the disagreeable odors and some of the mild irritants. In other subjects there was a change in blood pressure during the inhalation of the disagreeable and some or all of the mild irritants, which may be a rise in some instances and a drop in others. Wintergreen produced marked blood pressure changes in some subjects. All of the extremely irritating substances caused a change in blood pressure which was usually a rise. Aromatic spirits of ammonia always caused a rise in my subjects, while ammonia itself sometimes brought about a drop.

The respiratory tracings obtained from my anosmic are only in partial agreement with Malan's 30-year-old anosmic. The latter is reported as giving no respiratory changes from inhalations of violets, scatol and acetic acid. In my subject the respiratory and blood pressure responses to the strong irritants, excepting oil of mustard, and to most of the mild irritants were only slightly weaker than was the case in the normal subjects while all of the odors and xylol did not alter blood pressure or respiration.

Since the respiratory changes were practically the same in all of the anesthetized subjects and this reaction is identical to the reaction in rabbits, namely, inhibition caused by a shortening or a shortening and a slowing of the excursions, this inhibitory action is thought to be the respiratory reflex in man resulting from stimulation of the olfactory and trigeminal

endings in the nasal mucosa by the inhalants; while other alterations of respiration frequently obtained in unanesthetized subjects may result from psychic stimulation or other reflexes. It appears impossible to entirely exclude the aforesaid factors in unanesthetized subjects.

It would be of interest to know how and where sleep blocks the olfactory and the trigeminal respiratory reflexes.

SUMMARY AND CONCLUSIONS

In normal unanesthetized subjects the inhalation of the strong irritants produces marked depression and slowing or complete arrest of thoracic respiration. The thorax generally becomes much more contracted.

Mild irritants, disagreeable and agreeable odors cause either a considerably lowered inspiratory phase and an increase (sometimes no change or a decrease) in the rate of the excursions or a considerably deepened inspiratory phase and a decrease (sometimes no change) in the rate of the excursions. A few tracings show a combination of these two reactions. Frequently there is some effort toward deepened expirations in case of the disagreeable odors and mild irritants, where the response is much stronger than it is with the agreeable odors.

In normal unanesthetized subjects the systolic blood pressure is unaltered by inhalation of the mild agreeable odors. Disagreeable odors and mild irritants may or may not alter blood pressure and the change may be a rise or a drop. Strong irritants produce considerable change in blood pressure, usually a rise.

Respiration of the anosmic shows no changes from the inhalation of the agreeable and disagreeable odors or of wintergreen and xylol. Peppermint, menthol, eucalyptus, chloroform, ether, benzol, weak concentrations of the strong irritants and strong concentrations of the vapor from oil of mustard elicit a deepened inspiratory phase and a reduced rate of excursion. Strong concentrations of the very irritating substances, excepting oil of mustard, induce a considerably shortened inspiratory phase and some increased rate of excursion.

The changes in blood pressure of the anosmic during inhalations follow very closely those of respiration. Some mild irritants although not detected by the subject effect a change in respiration and blood pressure.

A long series of inhalations of various agreeable and disagreeable odors and mild irritants failed to awaken a sleeping subject or to produce any respiratory changes. The first inhalation of strong ammonia for over 20 seconds in one test evoked no respiratory response and did not awaken the subject.

Respiration in several subjects under light ether anesthesia responded to five inhalants as follows: Oil of cloves usually produced some depression or depression and slowing of the excursions, with one exception this depres-

sion affected only the inspirations. In those subjects where asafetida and xylol react, the effect is very similar though often weaker than oil of cloves, and the inhibitory influence of xylol is decidedly weaker than in rabbits. Wintergreen usually evoked a similar but much stronger inhibitory action than oil of cloves while strong concentrations of ammonia generally brought respiration to a standstill.

Blood pressure in anesthetized subjects presented so many inconstant and rapid changes that readings taken during the interval of an inhalation were of no value.

The inhibitory respiratory reaction obtained from inhalants in human subjects under anesthesia and in some records from non-anesthetized subjects is apparently the olfactory-trigeminal respiratory reflex from inhalants in man. This is identical to the olfactory-trigeminal reflex in rabbits. Different respiratory changes sometimes occurring in unanesthetized subjects may come from psychical stimulation.

BIBLIOGRAPHY

CROSLAND, GOODMAN AND HOCKET. 1926. Journ. Exper. Psychol., ix, 398.
MALAN. 1925. Arch. internat. de laryngol., iv, 666.
PONZO. Cited by MALAN.
RUSSI. Cited by MALAN.

STUDIES OF MUSCULAR EXERCISE UNDER LOW BAROMETRIC PRESSURE

IV. The Pulse Rate, Arterial Blood Pressure and Oxygen Pulse[1]

EDWARD C. SCHNEIDER and ROBERT W. CLARKE

From Wesleyan University, Middletown, Conn.

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It is the purpose of this paper to consider the changes in the circulatory reactions of physical exertion brought about by reductions in barometric pressure, down to 290 mm., which pressure is approximately equivalent to that of an altitude of 25,000 feet. All experiments were conducted in a low pressure chamber at pressures of 760, 535, 425, 350, and 290 mm. These pressures simulate altitudes of sea-level, 10,000, 15,000, 20,000, and 25,000 feet. A bicycle ergometer of the Benedict-Cady type provided the means for exactly controlled amounts of physical work, and the loads used were 2000, 4000, 6000, 8000, and 10,000 foot-pounds per minute. Physiologic observations were made prior to, during, and after the period of exertion.

The pulse. The experience of aviators and mountaineers has made it clear that at high altitudes a smaller amount of exercise is required to produce a given response of the pulse than at sea-level. Hingston (1925) observed, during the last expedition to Mount Everest, that, after the exercise of standing alternately on a chair and on the ground 5 times in 15 seconds, there was a marked increase in the pulse rate at all of the higher altitudes. The rate rose at sea-level from 72 and 84, and at an altitude of 21,000 feet from 120 to 144. Barcroft and collaborators (1923), in their account of the Andes Expedition, state that the cardiac response to exercise at an altitude of 14,200 feet is very similar to that found in cases of so-called "irritable heart." The rate is excessive in exercise, continues to be excessive after the exertion has ceased, and usually requires a much longer period of rest before returning to the pre-exercise rate.

In order to establish a basis of comparison for the effect of a reduction in barometric pressure on physical exertion we have first determined the normal response of our seven subjects at sea-level. Since the circulatory response may be influenced by the speed of movement, the resistance

[1] The experimental data given in this paper were obtained at the School of Aviation Medicine, Mitchel Field, L. I., N. Y.

encountered, and the condition of the individual (Bowen, 1904), we were careful to maintain a constant state of all factors except the load of work. The pedaling of the bicycle ergometer was always done at the same rate, 70 revolutions per minute. In order to avoid an unnecessary rise in body temperature two electric fans were allowed to play on the subject while he worked.

Under the conditions of our experiments the pulse rate at sea-level pressure was approximately a linear function of the exertion. The data for C. R. J., which have been plotted in figure 1, show that at sea-level this linear relationship was maintained up to and including a load of 10,000 foot-pounds. While C. R. J. was sitting at rest on the ergometer his average pulse rate was 75; with a load of 2000 foot-pounds the rate was 105; with 4000 foot-pounds, 132; with 6000 foot-pounds, 154; with 8000 foot-pounds, 177; and with 10,000 foot-pounds, 198. The successive increases in rate were 30, 27, 22, 23, and 21 respectively.

When the load of work becomes excessive the heart fails to keep pace with the demands made upon it and thus breaks the linear relationship. This is illustrated in the case of R. W. C. (table 1) who showed average increases in pulse rate of 24, 29, 29, and 31 with loads of 2000, 4000, 6000, and 8000 foot-pounds and then with a load of 10,000 foot-pounds had a further increase of only 15 beats. Another example is that of A. L. H. whose pulse rate with a load of 2000 foot-pounds was 119; with 4000 foot-pounds, 143; with 6000 foot-pounds, 168; with 8000 foot-pounds, 189; and with 10,000 foot-pounds, only 195. Thus after having had an average increase of 25 beats with successive equal additions to the load, the increase in the pulse dropped to only 6 when the load was augmented from 8000 to 10,000 foot-pounds.

From our data on physical exertion at sea-level, we may conclude that the pulse frequency will roughly maintain a linear relationship with work as it increases up to a certain load; and that beyond this point the pulse will respond to the additional load in a lesser degree than theretofore. Furthermore it seems probable that eventually a load may be reached that will not cause further acceleration of the pulse.

Judging from the experience of mountaineers and aviators, a marked irritability of the heart during work at a reduced barometric pressure might be expected. This was not, however, found to be true for any of our subjects. The anoxemic influence was always roughly proportional to the reduction in pressure, hence the extent of pulse acceleration was also roughly predictable. Consequently, plotted lines (fig. 1) of the pulse frequency for equal increments in the load of work at the several barometric pressures are approximately parallel with those for the reduced pressures above that of sea-level pressure.

Summaries of the results of all experiments on three subjects are given

in table 1. These data are typical for our group of seven subjects. Of these, H. M. showed a linear relationship between the pulse frequency and load of work at 760 mm. (sea-level) for all loads investigated; but when he was under a reduced barometric pressure this relationship was not so evident, because the resting pulse was not accelerated in proportionate amount at each reduction of pressure. Had he, however, been given more of the moderate loads of work, the linear relationship would certainly have been demonstrated. This is indicated by the fact that the plotted curves for loads of 2000 and 4000 foot-pounds at all low pressures were parallel

TABLE 1

The average pulse rate during work at different barometric pressures

BAROMETER	AT REST	2000 FOOT-POUNDS	4,000 FOOT-POUNDS	6,000 FOOT-POUNDS	8,000 FOOT-POUNDS	10,000 FOOT-POUNDS
			H. M.			
mm.						
760	78	101	132	156		
535	84	113	145	162		
425	87	124	153	166		
350	87	131	161	174		
290	102	144				
			R. W. C.			
760	74	98	127	156	187	202
535	84	110	143	167	189	
425	87	129	163	180		
350	97	148	176	195		
290	104	144				
			C. R. J.			
760	75	105	132	154	177	198
535	86	117	148	168	186	198
425	99	129	162	177	183	
350	111	144	171	174		
290	123	159	165			

with the sea-level curve for these loads. The increase in the pulse rate for the two loads of work, 2000 and 4000 foot-pounds, over that for work done at 760 mm. was, at a barometric pressure of 535 mm., 12 and 13 beats respectively; at 445 mm., 23 and 21 respectively; and at 375 mm., 30 and 29 respectively. The next higher load, 6000 foot-pounds, was excessive for H. M., hence the effect of the reduction in barometric pressure was less in evidence. The increase in pulse rate over that at sea-level for this load was only 6, 4, and 8 beats at the successive reductions of barometric pressure.

The influence of low barometric pressures on the pulse rate during exertion was most thoroughly studied on C. R. J. He was our strongest subject and willingly carried loads up to 10,000 foot-pounds while under reduced pressures. The averages of all experiments on him are given in table 1 and are plotted in figure 1. The lines for work done at each reduction of barometric pressure are roughly parallel for the lighter loads and thus show the linear relationship between the pulse rate and load of work to be maintained at all pressures down to 290 mm. The lower the barometric pressure, however, to which one is subjected, the lighter is the load at which the linear relationship breaks down. Thus at 750 mm. this relationship was maintained for all loads up to 10,000 foot-pounds; while at 535 mm. it was roughly maintained to 8000 foot-pounds; at 425 mm., to 6000 foot-pounds; at 350 mm., to 4000 foot-pounds; and at 290

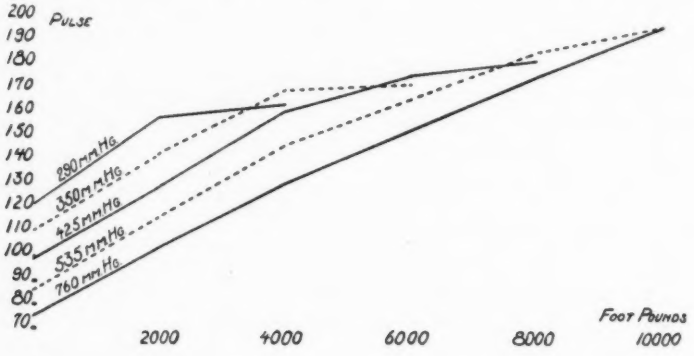

Fig. 1. Pulse frequency during work at low barometric pressures

mm., to only 2000 foot-pounds. The break in the linear relationship usually appears suddenly. Thus, at a barometric pressure of 535 mm., the low pressure increase in pulse rate over the rate for the same load at sea-level for the successive loads up to 10,000 foot-pounds was 12, 16, 14, 9, and 0, respectively; at a pressure of 425 mm., 24, 30, 23, and 6 respectively; and at 350 mm., 39, 39, and 3 respectively.

The rate of augmentation of pulse frequency. During muscular exertion there is usually a rapid primary rise, for from one to four minutes, in the pulse frequency at the beginning of work. The frequency may then remain constant for a while and later retard or again augment. This is well illustrated in our data for R. W. C., a part of which are given in table 2. The resting pulse rate of R. W. C., at a barometric pressure of 760 mm., averaged 74. At sea-level pressure, when he merely pedaled the unloaded ergometer, his pulse frequency was not altered during a period

of eight minutes; but, with a load of 2000 foot-pounds, it rose to 96 during the first minute of work, remained at this during the next five minutes, and then slowly accelerated to 104 during the last three minutes of work. With a load of 4000 foot-pounds the frequency rose to 112 during the first minute; then increased more gradually to 132 during the next five minutes, after which it retarded to 128. A load of 6000 foot-pounds accelerated the pulse to 116 during the first minute and thereafter gradually and steadily raised it to 156 during the remaining seven minutes of work.

A reduction of barometric pressure does not materially alter the curve of response during physical exertion. The data for R. W. C., given in table 2, are typical for all of our seven subjects. They show that the influence of anoxemia is a constant factor which is manifest in approximately equal degree for all loads of work. With a load of 6000 foot-pounds R. W. C.'s

TABLE 2

R. W. C., pulse rate at the end of each minute of work

BAROMETER	REST	1ST	2ND	3RD	4TH	5TH	6TH	7TH	8TH
Load of work, 2000 foot-pounds per minute									
mm.									
760	74	96	96	96	98	96	100	102	104
535	84	104	104	104	104	108	112	110	110
445	92	108	112	112	116	114	116	116	116
350	104	136	140	140	140	140	136	136	132
Load 4000 foot-pounds									
760	74	112	120	124	128	132	128	128	128
535	88	116	140	134	144	148	152	144	144
425	92	132	144	148	152	156	160	160	165
350	96	128	148	148	172	172	168	176	176

pulse rate accelerated minute by minute throughout a 4-minute period as follows: at a pressure of 760 mm., from 84 to 164; at 535 mm., from 85 to 167; at 425 mm., from 100 to 180; and at 350 mm., from 105 to 195

While our plan of work called for comparatively short periods of exertion, it seemed desirable to determine how a longer period of work would affect the pulse rate. For this purpose we had 2 men each twice carry a load of 3000 foot-pounds for 30 minutes at a pressure of 380 mm. (altitude 18,000 ft.). In the two experiments on R. W. C. the pulse rate gradually rose from 74 to 164 in 11 and 16 minutes respectively, and then remained almost constantly at that level during the remainder of the period of work. At 760 mm. the pulse accelerated from 68 to 100 in two minutes, and then more gradually to 110 by the 13th minute, after which it fluctuated around 110. Experiments on L. M. T. gave similar results. At 760 mm. the pulse

rate in 3 minutes accelerated from 72 to 108 and fluctuated about that as a mean for the balance of the work period. In the first experiment at 380 mm., the pulse frequency rose rapidly from 84 to 124 in 2 minutes, and then gradually to 132 during the next 11 minutes and remained there. In the second experiment at 380 mm., the pulse rate rose from 72 to 105 in 2 minutes, then more gradually rose to 130 to remain there. These longer experiments, like the short periods of work, give no evidence that the lack of oxygen experienced at low barometric pressures progressively affects the irritability of the heart. The only change that has been found is that, with any given load of work, the heart beats more rapidly at low barometric pressures than it does when the same load of work is carried at the sea-level pressure.

Post-exercise retardation of the pulse. Our data on the decline in the pulse rate after exertion were in large part obtained by making a 20-second count the last 20 seconds of each minute, but in several series of experiments the pulse was counted in 15 second intervals throughout the first 3 minutes following work and thereafter every half minute. As a rule the time of return to the pre-exercise rate after short periods of work, 6 to 8 minutes, was approximately the same at sea-level and low barometric pressures. After carrying a load of 2000 foot-pounds for 6 minutes, the return was made in 4 subjects within 2 and 3 minutes at all pressures to which they were subjected. With a load of 4000 foot-pounds, three of the men invariably made the return within 3 and 4 minutes at all pressures. One subject, C. R. J., had a delayed return when under pressures of 535 and 425 mm., but at 350 and 290 mm. the return was made as soon as at sea-level. In one experiment in which he did 4000 foot-pounds of work per minute for 6 minutes at a pressure of 290 mm., equivalent to an altitude of 25,000 feet, the pulse rate returned from 165 to the pre-exercise rate of 130 in 5 minutes; after which it went subnormal, dropping to 120 by the seventh minute, to 117 by the eleventh minute, and to 114 at the twentieth, where it remained during the balance of the observation period of 30 minutes.

Since the pulse rate returns to the pre-exercise rate in approximately the same time at all barometric pressures studied, it follows that the decline in pulse rate is the more rapid the lower the pressure at which work is done.

Following longer work periods the return of the pulse to the pre-exercise rate is not as complete under low barometric pressure as is the case with the shorter periods of work. After R. W. C. did 3000 foot-pounds of work for 30 minutes at sea-level, the pulse rate returned to normal in 22 minutes; but when the same amount of work was done at 380 mm. barometric pressure, the pulse was still 10 beats above the pre-exercise rate 48 minutes after work ceased. L. M. T. also experienced a more lasting effect at the low barometric pressure. At the normal barometric pressure, the pulse

rate returned to the pre-exercise rate in 16 minutes; but at a barometric pressure of 380 mm., it was still 12 beats above the pre-exercise level 30 minutes after an exercise period of 30 minutes.

Our data bring out clearly 1, that while a linear relationship is maintained between the pulse frequency and the load of work at low barometric pressures, this relationship is broken with a lighter load at low pressures than at sea-level. This break, furthermore, appears before a maximal response has been made by the heart, occurring with lighter loads the lower the barometric pressure. 2. That for any load, under that at which the pulse fails to accelerate at the normal rate, the frequency of the pulse is always greatest at the low barometric pressures and is roughly proportional to the reduction in pressure. 3. That the curves of the rate of acceleration during the carrying of any load of work are similar at the several pressures we have studied. 4. That after short periods of exertion the pulse frequency returns to the pre-exercise rate in about the same time at all pressures, but that after more prolonged work it may return more slowly at the low barometric pressures.

It is evident that some of these observations are not in accord with results obtained by a study of men at high altitudes of mountains. The differences, very likely, may be explained by the physical condition of the subjects studied and by mountain sickness or the stage of acclimatization.

The arterial blood pressure. Before we consider the arterial blood pressure changes of physical exertion at reduced barometric pressures it may be well to recall some earlier observations on the arterial blood pressure of men at low atmospheric pressures. Lutz and Schneider (1919) showed that the systolic pressure of the majority of all men when at rest was unaffected throughout the entire period of exposure to a reduced barometric pressure, but that approximately 25 per cent of all cases experienced at first a slight rise and later an even more moderate drop in the pressure. In a still smaller percentage of men the reduction of barometric pressure caused a slight but progressive fall in the systolic blood pressure throughout the entire period of exposure. It was found that the resting diastolic pressure sooner or later began to fall in 70 per cent of all cases until a new level was reached and held. The fall in diastolic pressure, at 380 mm. barometric pressure, averaged 12 mm. and ranged from 4 to 28 mm. In some men this pressure was unaffected by the reduction in barometric pressure.

The effects of low barometric pressure on the arterial blood pressure during physical work have not received much attention. Schneider, Cheley, and Sisco (1916) found on Pike's Peak, barometer 450 mm., altitude 14110 feet, that the arterial pressures were higher after a given form of work at the high than at the low altitude. The influence of the lowered barometric pressure was the more pronounced the more vigorous the exertion. Fur-

thermore the reaction was most conspicuous during the first days of residence, and became decidedly less, but did not wholly disappear, after a residence of two weeks. The increase in diastolic pressure was comparatively slight. The general conclusions arrived at were that the influence of a low barometric pressure, during and immediately after exertion, reveals itself in a more marked rise in all the arterial pressures than usually occurs at ordinary barometric pressure, and in a greater delay in their return to normal after cessation of work.

If a reduction in barometric pressure does modify the arterial blood pressure changes of physical exercise, it is possible that its action may be manifest in one or more of a variety of ways. Within certain limits of physical effort, the systolic arterial blood pressure increases. Gillespie, Gibson, and Murray (1925) found the rise in this pressure to be approximately proportional to the load, but not in a definite linear relationship, because of many individual variations. It is to be expected from the results obtained by Schneider, Cheley, and Sisco that successive reductions in the barometric pressure should affect the degree of rise in the systolic pressure when uniform increments are added to the load of work. Bowen (1904) observed, for men at sea level, that normally there is rapid rise in the systolic pressure at the very onset of exercise, succeeded by a more gradual further rise to a maximum, which is reached within five to ten minutes from the beginning of work. Lowsley (1911), at sea-level, found that the diastolic pressure also rises in exercise, while Erlanger and Hooker found that very moderate exertion may diminish this pressure. Frequently, as work is continued, both of these arterial pressures may show a gradual fall. After the conclusion of work there is a rapid fall in both pressures to normal or subnormal. Immediately after the cessation of work the systolic pressure has been shown by Cotton, Lewis, and Rapport (1917) to be little, if at all, above the normal resting level. It then again rises rapidly, in from 20 to 60 seconds, to near the level held during the work period, after which it gradually falls. The diastolic pressure shows a similar series of changes.

From the above recital of the changes in the arterial blood pressure during and after exercise, it is evident that a reduction in barometric pressure may influence the extent and time of the rises in the systolic and diastolic pressures; the shape of the curve of changes during exercise; the extent and duration of the early post-exercise drop in the pressures; and the time required for the final restoration of the pressures to the pre-exercise level.

Our arterial blood pressure data are fairly complete for 6 subjects who pedaled the bicycle ergometer without resistance in the magnets, and then carried loads of 2000 and 4000 foot-pounds at barometric pressures of 760, 535, 425, and 350 mm. A small amount of data is available for loads of 6000 and 8000 foot-pounds at some of these pressures.

The arterial blood pressures were determined by the auscultatory method with a Tycos sphygmomanometer. The systolic pressure was read at the beginning of the first phase and the diastolic pressure at the fourth phase; that is, at the dulling of the intense sound of the third phase.

Each of our subjects, at 760 mm., showed a rise in the systolic pressure with each increment to the load of work, beginning with 2000 foot-pounds. Pedaling without resistance always caused either no change or a fall in the systolic pressure.

The influence of a reduction of barometric pressure on the systolic blood pressure during work was not the same for all of our subjects. In three of the men the maximum systolic pressure tended, with moderate loads of work, to be greater the lower the barometric pressure; while another showed

TABLE 3

Systolic pressure of W. S. R. during 8 minutes of work

LOAD	BAROMETER	REST	1ST	2ND	3RD	4TH	5TH	6TH	7TH	8TH
foot-pounds	*mm.*									
Pedaling (no load)	760	112	116	116	116	114	114	114	112	112
	535	108	106	106	108	106	106	108	108	108
	445	106	104	104	104	104	104	104	104	102
	350	104	104	104	104	106	106	106	106	106
2,000	760	108	116	120	120	118	120	122	120	120
	535	110	106	120	122	118	116	120	120	120
	445	106	108	110	118	118	118	122	120	124
	350	106	114	110	116	120	120	124	130	136
4,000	760	116	120	122	122	124	126	128	132	132
	535	116	120	120	128	128	126	128	134	145
	445	114	129	126	132	132	136	140	140	148
	350	118	120	124	140	144	144	144	146	142

the low barometric effect by the increase in arterial pressure, but the change was variable with equal decrements of the barometer. In two of the men the usual rise in the systolic pressure of exercise was not influenced by the fall in barometric pressure.

The data for the systolic pressures obtained from W. S. R. are given in table 3. His work period was always 8 minutes long. When he merely pedaled the bicycle ergometer at the rate of 70 revolutions per minute, his systolic pressure, at a barometric pressure of 760 mm., at once rose from 112 to 116 mm. and then gradually returned to 112 mm.; while at three reductions of barometric pressures the systolic pressure was unaffected by this amount of work. With a load of 2000 foot-pounds the systolic pressure rose at approximately the same rate and degree at barometric pressures

of 760 and 535; but when the barometer was 425 mm. this load of work caused the systolic pressure to gradually rise 6 mm. more than at the other levels and when the barometer stood at 350 mm. this load slowly caused the systolic pressure to rise 12 mm. more than it had at 425 mm. Hence, with a load of 2000 foot-pounds, the reduction in barometric pressure is without effect until it is lowered to less than 535 mm. A further reduction, however, in the barometer then causes a distinct augmentation of the systolic pressure.

With a load of 4000 foot-pounds, the low oxygen effect is brought out by a more moderate reduction of barometric pressure. The effect is then manifest when the barometer is at 535 mm. At 760 mm. the systolic pressure gradually rose from 116 to 132 mm.; while at 535 mm. it rose from 116 to 145 mm. in 8 minutes.

The experiments on W. S. R. clearly indicate that the influence of a reduction in barometric pressure during work may be manifest by a higher systolic pressure, and by a more rapid rise in the arterial blood pressure. These effects were well shown by S. S. Y. when he did 4000 foot-pounds of work per minute for 6 minutes. At 760 mm. his systolic pressure rose to 130 mm. in 6 minutes; at 535 mm., to 154 mm.; and at 425 mm., to 162 mm. in the same time; while at 350 mm. it rose to 170 mm. in 4 minutes.

C. R. J. carried a load of 8000 foot-pounds for 3 minutes at barometric pressures of 535, 425, and 350 mm. with the following changes in his systolic pressure: at 535 mm. it rose from 110 to 180 mm.; at 425 mm., from 114 to 192 mm.; and at 350 mm., from 118 to 202 mm.

After exercise the systolic pressure usually returned to the pre-exercise level in approximately the same time at the various barometric pressures. This was true for three of our subjects for all loads carried at barometric pressures down to 350 mm., and for another for pressures down to and including 425 mm. The latter, however, showed a delayed return of from 2 to 6 minutes for loads of 2000 and 4000 foot-pounds when at 350 mm. C. R. J. showed a marked delay in the return of the systolic pressure after heavy loads of work. Thus, after he carried a load of 8000 foot-pounds for three minutes, the return was made in 5.5 minutes at 760 mm., in 19 minutes at 535 mm., and was not back to the pre-exercise rate in 25 minutes at 425 and 350 mm. Another subject, W. S. R., who was only studied for a load of 4000 foot-pounds, had a delayed return at all the low pressures at which he worked. His return was made in 1.75 minutes at 760 mm., in 9 minutes at 535 mm., in 10 minutes at 425 mm., and in 17 minutes at 350 mm.

The temporary fall in the systolic pressure, that immediately follows a work period, was not clearly influenced either in extent or duration by a reduction in barometric pressure.

Diastolic pressure. The effect of reductions in barometric pressure is

illustrated in table 4 by the data of a series of studies made on W. S. R. It is evident that there is no influence with moderate reductions of pressure and light loads of work. Ordinarily the diastolic pressure is unchanged or shows a slight rise during work. When W. S. R. merely pedaled the unloaded ergometer, his diastolic pressure remained quite uniform until a barometric pressure of 350 mm. was used. Under that barometer the diastolic tension decreased slightly during the last 3 minutes of an 8-minute work period. With loads of 2000 and 4000 foot-pounds the fall in the diastolic tension appeared at a barometric pressure of 425 mm.

It was first thought that the drop in diastolic pressure during work at a low barometric pressure was proportional to the reduction in barometric pressure, but we were unable to demonstrate this. That the influence

TABLE 4

Diastolic pressure of W. S. R. during 8 minutes of work

WORK	BAROMETER	REST	1ST	2ND	3RD	4TH	5TH	6TH	7TH	8TH
foot-pounds	*mm.*									
No load	760	74	74	74	74	74	72	74	74	72
	535	70	74	74	72	74	74	74	74	74
	425	74	74	74	72	72	72	72	72	72
	350	74	74	74	74	70	72	68	64	66
2,000	760	74	74	74	74	74	72	70	74	74
	535	70	70	70	70	72		70	70	68
	425	70	66	64	60	62	60	60	60	
	350	70	70	68	70	58	56	50	50	50
4,000	760	76	70	72	72	72	76	72	74	72
	535	76	78	78	74	76	74	76	74	70
	425	74	70	70	66	66	60	64	60	60
	350	76	70	60	63	62	60	60	54	56

appears at a smaller reduction in barometric pressure as the load increases is clear from the data of W. S. R. The heaviest load for which data are available is that of 8000 foot-pounds, which load was carried for 3 minutes by C. R. J. at 535, 425, and 350 mm. At 535 mm. his diastolic pressure fell from 80 to 70 mm.; at 425 mm., from 74 to 40 mm.; and at 350 mm., from 75 to 40 mm.

Not only does the diastolic pressure show a greater fall during exercise at a low than a high barometric pressure, but after work there is a greater drop and a slower return to the pre-exercise level. Here again with moderate efforts and reductions of barometric pressure no clear anoxemic influence is seen. Data obtained on W. S. R. illustrate these points. With the very easy work of merely pedaling the non-electrified ergometer, the post-exer-

cise diastolic pressure showed a slight fall and was quickly restored at 760, 535, and 425 mm. barometric pressure; while at 350 mm. the diastolic pressure fell to 60 mm. immediately after exercise and required 240 seconds to be restored to 74 mm., the pre-exercise level. With a load of 2000 foot-pounds, no after-effect on the diastolic pressure occurred at 760 mm.; while at 535 mm. it fell from 68 to 60 immediately after exercise and was back to 70 in 45 seconds; at 425 mm. it was restored from 60 mm. in 30 seconds; but at 350 mm. it fell as low as 30 mm. and required 360 seconds for the return to the pre-exercise level. With a load of 4000 foot-pounds the influence of a reduction in barometric pressure appeared at a higher pressure and was more lasting. Thus at 760 mm. the diastolic pressure fell to 62 and was restored to the pre-exercise level in 45 seconds; at 535 mm., it fell to 40 mm. and required 135 seconds for the return; at 425 mm. it fell to 50 mm. and was restored in 420 seconds; and at 350 mm. it fell to 30 mm. and required 960 seconds to return to the pre-exercise level.

Work at a simulated altitude of 25,000 feet. The lowest barometric pressure under which we had subjects work was comparable to that of an altitude of 25,000 feet. At this pressure loads of 2000 and 4000 foot-pounds were carried for from 5 to 8 minutes without harm or even great discomfort to the worker. In view of the common belief that the capacity for work is greatly reduced at high altitudes, it is of special interest to review our observations on 3 men who worked at this low pressure. That men acclimatized to high altitude are capable of a fair degree of physical effort has been demonstrated by the three expeditions to Mount Everest. Somervell (1923) wrote: "Below the North Col (23,000 feet), I took three breaths to a step, while at 26,000 feet I was taking five complete respirations; but as long as I was walking slowly enough I experienced no distress or discomfort." "At the height of 26,000 feet, I took my pulse (which was 180) and my respirations (which were 50 to 55 to the minute); but withal one felt perfectly comfortable even though these abnormal physiological conditions were present." In the last expedition Norton (1925), while at 28,000 feet, ascended only about 80 feet in an hour's climb.

At a barometric pressure of 290 mm. (altitude 25,000 feet) our subjects, who were in no sense acclimatized, soon became, while at rest, quiet and listless and sometimes even groggy. They were very cyanotic in the lips and ears, and showed a dark background in the skin of the face and neck. Soon after they began to work the color showed improvement, becoming less blue and decidedly more pink. Within two minutes after cessation of work the blue color began to reappear and was soon as dark as before exercise. The frequency of breathing, when a load of 2000 foot-pounds was carried, ranged between 23 and 30 breaths per minute; while one subject, who did 4000 foot-pounds of work, breathed 35 times a minute. In some of the experiments the minute volume of breathing was determined.

With a load of 2000 foot-pounds, it ranged between 34 and 55 liters; and with a load of 4000 foot-pounds, it reached as much as 82 liters.

None of the men felt uncomfortable during the work, although at times they were conscious of their breathing and gave evidence of some respiratory distress. That the mind was somewhat affected was evidenced by the fact that, after the entire experiment was finished, it was impossible to recollect whether the final work period had been wholly carried out according to the plan. No bad after-effects were observed.

The pulse rate of our subjects did not rise extraordinarily high during work at the barometric pressure of 290 mm. On two occasions of 5 minutes each, H. M. pedaled the ergometer at the rate of 70 revolutions a minute without the electro-magnetic resistance. The first time the pulse rate reached 123 and the second 132 beats per minute. R. W. C. experienced, during the same amount of work, a rise to 123 and then a retardation to 105. With a load of 2000 foot-pounds for 5 minutes, H. M.'s pulse went to 144; R. W. C.'s to 150; and C. R. J.'s to 159. With a load of 4000 foot-pounds, C. R. J.'s pulse rate rose to 165. In each case, where the return of the pulse rate was followed after exertion, the pre-exercise rate was reached in from 45 seconds to 5 minutes. But in every instance there was a further retardation until the rate was from 8 to 16 beats below the pre-exercise level. This lower rate was then maintained throughout the remainder of the period of observation.

We have only one good record of the arterial blood pressure changes during work under a barometer of 290 mm. In this experiment R. W. C. carried a load of 2000 foot-pounds for 8 minutes. During the first three minutes of work, he breathed as his respiratory center dictated; but as he felt some respiratory distress and breathed gaspingly, he then began to breathe in unison with his pedaling of the bicycle ergometer. With this forced type of breathing, which corresponded in a way with the usage of the Mount Everest Expeditions, he showed improvement in color, pulse rate, and blood pressure. Prior to being subjected to a reduced barometric pressure, his arterial systolic blood pressure was 116 and the diastolic pressure 76 mm. Under the barometric pressure of 290 mm., as he sat quietly, the arterial pressures had lowered to 106 and 58 mm. respectively. At the end of the first and second minutes of work, these pressures were 106 and 50; and at the end of the third minute, 110 and 50 respectively. At the end of the fourth minute, after one minute of forced breathing, they were still 110 and 50; but thereafter each began to rise. At the end of the fifth minute they were 110 and 56; at the sixth, 130 and 60; at the seventh, 140 and 64; and at the eighth, 135 and 66.

The pulse rate, in this experiment on R. W. C., rose from 108 to 156 during the first three minutes of work. The forced breathing then gradually retarded it minute by minute to 152, 144, 128, 124, and 120. After

the work period, the rate retarded in 45 seconds to 100 and remained thereabout for the next 10 minutes, at which time the systolic pressure was 110 mm. and the diastolic pressure 70 mm. Unlike other reduced barometric pressure experiments, the arterial diastolic pressure did not show the great drop immediately after the exertion.

Our experience shows that healthy unacclimatized men can do moderate amounts of physical work at a barometric pressure of 290 mm. for a short time without much distress and without harm. A beneficial effect of forced

TABLE 5

The oxygen pulse during rest and work

BAROMETER	REST	2,000 FOOT-POUNDS	4,000 FOOT-POUNDS	6,000 FOOT-POUNDS	8,000 FOOT-POUNDS	10,000 FOOT-POUNDS
			H. M.			
mm. Hg						
760	3.6	9.4	11.6	12.1		
535	2.9	8.1	10.3	11.4		
445	3.1	7.1	9.4	10.9		
350	2.9	6.4	8.5	8.6		
290	3.0	5.4				
			R. W. C.			
760	3.5	8.5	10.7	12.1	14.2	15.3
535	3.1	7.7	9.5	11.5	12.5	
445	2.5	6.2	7.8	9.3		
350	3.0	5.2	7.3	7.9		
290	2.9	5.7				
			C. R. J.			
760	4.2	8.7	11.3	11.6	13.7	15.8
535	3.7	7.9	10.3	9.0	12.7	12.1
445	3.2	7.6	9.3	10.7	12.5	
350	3.2	6.2	7.9	8.9		
290	2.9	4.6	6.6			

breathing is shown in the circulatory response of R. W. C., when he was working under that low pressure.

The oxygen pulse. The oxygen pulse has been defined by Henderson and Prince as the amount of oxygen consumed by the body from the blood of one systolic discharge of the heart. Its value is determined by dividing the amount of oxygen absorbed per minute by the number of heart beats in a minute. From a study of the oxygen pulse for work done under a low barometric pressure, some knowledge can be gained of the handicap experienced by the body when it is placed under this condition.

We have shown in another paper that the amount of oxygen used by the

muscles during work at a reduced barometric pressure is decreased approximately proportionately with the fall in barometric pressure. This suggests that the amount of oxygen delivered to the tissues per unit volume of blood falls off as the barometric pressure is lowered. Therefore, unless the heart should greatly increase its output of blood per beat, while the individual is under a reduced barometric pressure, it is to be expected that the oxygen pulse will fall off for any given load of work at each successive stage of reduction in the barometric pressure. Our data clearly show this to be true.

We have given the oxygen pulse for three of our subjects in table 5. The amount of oxygen delivered to the body by each heart beat in H. M., when a load of 2000 foot-pounds was carried at 760 mm. (sea level), was 9.4 cc. At each of the simulated altitudes of 10000, 15000, 20000, and 25000 feet, the delivery fell off approximately one cubic centimeter of oxygen; so that at the simulated altitude of 25000 feet, the delivery per heart beat was 4 cc. less than at sea level. That the effect of a reduction in barometric pressure is approximately the same for all loads of work is indicated by the decrease in H. M.'s oxygen pulse at a barometric pressure of 350 mm. (20,000 feet) for loads of 2000, 4000, and 6000 foot-pounds. The decrease in the oxygen pulse was 3 cc. for a load of 2000 foot-pounds, 3.1 cc. for one of 4000 foot-pounds, and 3.5 cc. for one of 6000 foot-pounds.

The oxygen pulse, ordinarily, during work at sea level, increases with the acceleration of the heart rate; and the heart rate, of course, accelerates proportionately to the load of work. From our data given in table 5, it is evident that, at each reduction of the barometric pressure, the oxygen pulse still augments with the load of work; but this increase is not as great at reduced pressures as it normally is at sea level. When the work curves for the oxygen pulse are plotted for several barometric pressures, the curve at each successive reduction in pressure falls under the one of the next preceding higher barometric pressure. These curves for our data obtained from H. M. and R. W. C., are roughly parallel for the several barometric pressures; while those for C. R. J. show some degree of irregularity, they, nevertheless, also support our conclusion as stated above.

These data for the oxygen pulse bring out clearly the fact that the heart performs its function of delivering oxygen to the tissues under a great handicap, when work is done at reduced barometric pressures; and these data also indicate that the capacity for work is reduced because less oxygen is delivered to the tissues.

SUMMARY

At a barometric pressure of 760 mm. Hg the pulse frequency roughly maintains a linear relationship with the load of work until it becomes an

over-load, the pulse then fails to accelerate in the same proportion when the load is further increased.

Anoxemia causes a greater increase in the frequency of the heart beat at a reduced barometric pressure than at 760 mm. Hg. A marked irritability of the heart in work is not present at reduced barometric pressures. Plotted lines of the frequency of the pulse during work under low barometric pressures are approximately parallel with the curve for 760 mm., and show the increased frequency of the heart beat in work to be roughly proportional to the reduction in barometric pressure. The lower the barometric pressure the smaller is the load at which the pulse frequency fails to maintain the linear relationship with the increase in the load of work.

A reduction in barometric pressure does not materially alter the curve of the rate at which the pulse frequency accelerates during physical work.

After short periods of work the pulse frequency returns to the pre-exercise level in about the same time at all pressures studied; but, after more prolonged work, it returns more slowly at low barometric pressures.

A reduction of barometric pressure does not influence the systolic arterial blood pressure of all men in the same way. This blood pressure usually rises higher and more rapidly with a given load of work the greater the reduction in barometric pressure. The return of the systolic blood pressure to the pre-exercise level is usually made equally promptly after light loads but after heavy loads of work may be much delayed at low barometric pressures.

When a reduction of barometric pressure influences the diastolic arterial blood pressure of work it causes a fall in this pressure. With very light loads of work the effect is not seen until the barometric pressure is reduced to 350 mm., but with heavier loads the effect is already evident at 535 mm. After exercise at low barometric pressures the diastolic blood pressure falls still lower and returns to the pre-exercise level very slowly.

The oxygen pulse for any given load of work falls off with each successive reduction of barometric pressure. The extent of the decrease is unaffected by the load, but is roughly proportional to the reduction in barometric pressure.

Healthy unacclimatized men can do a moderate amount of physical work at a barometric pressure of 290 mm. (equivalent to an altitude of 25,000 feet) for a short time without much distress and without harm. Forced breathing, while one is at work at a pressure of 290 mm., improves the circulatory responses and well-being.

BIBLIOGRAPHY

BARCROFT, J., C. A. BINGER, A. V. BOCK, J. H. DOGGART, H. S. FORBES, G. HARROP, J. C. MEAKINS AND A. C. REDFIELD. 1923. Phil. Trans. Roy. Soc. London, Series B, ccxi, 351.

BOWEN, W. P. 1904. This Journal, xi, 59.
COTTON, T. F., T. LEWIS AND D. L. RAPPORT. 1917. Heart, vi, 269.
ERLANGER, J. AND D. R. HOOKER. 1904. Johns Hopkins Hosp. Repts., xii, 145.
GILLESPIE, R. D., C. R. GIBSON AND D. S. MURRAY. 1925. Heart, xii, 1.
HINGSTON, R. W. G. 1925. The fight for Everest. London, 243.
LOWSLEY, O. S. 1911. This Journal, xxvii, 446.
LUTZ, B. R. AND E. C. SCHNEIDER. 1919. This Journal, l, 228.
SCHNEIDER, E. C., G. E. CHELEY AND D. L. SISCO. 1916. This Journal, xl, 380.
SOMERVELL. 1923. The assault on Mount Everest. 1922.

PHYSIOLOGIC STUDIES ON THE MOTOR ACTIVITIES OF THE STOMACH AND BOWEL IN MAN

WALTER C. ALVAREZ

From the Division of Medicine, The Mayo Clinic, Rochester, Minnesota

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The studies here described have been made at intervals during the last fifteen years whenever suitable material came to hand. I have delayed publication in the hope of securing more cases and more positive results, but it seems justifiable now to put on record what has been done if only that it may stimulate hospital internes and others to take advantage of the opportunities that from time to time arise in their work. It is so easy to pass a balloon into the human stomach that many workers have studied the movements of that organ, but strange to say, few have ever passed balloons into fistulas of the bowel and, so far as I know, no one has studied and compared the rhythmic and other properties of muscle excised from several parts of the digestive tract of man. Such work is much needed, and will have to be done before the results obtained in animals can be applied with confidence to the problems of human physiology and pathology.

A few men with the help of the barium meal and the fluoroscope, have counted the number of rhythmic contractions in the bowel of man, usually jejunum, and have reported rates of about ten a minute. I too have made many such counts in duodenum, jejunum, and terminal ileum and usually have found the rate about ten a minute. I could never be sure of a gradient in rate down the bowel similar to that which can be demonstrated so easily in animals. In one woman the rate in the duodenum was as low as six a minute.

Ganter, Weitz and Vollers using balloons passed through the esophagus and stomach into the small bowel found rates of from ten to twelve a minute. Similar rates were found also in a loop from the orad part of the jejunum transplanted under the skin of the chest to take the place of a stenosed esophagus; at times, however, the rate dropped to from five to six a minute. Plant and Miller found four contractions a minute in the ileum.

In a woman with the abdominal wall so thin that I could see the bowel directly, the rhythmic contractions came about ten times a minute, and in another, with an enormous ventral hernia, loops of what was thought to be ileum contracted from seven to nine times a minute. As will be seen

later, the rate of rhythmic segmentation in man varies suddenly from time to time much as it does in the cat and dog. In the rabbit and white rat it is much more constant.

STUDIES ON THE GASTRO-INTESTINAL MUSCLE OF AN EXECUTED MAN. In 1916 through the kindness of Dr. L. L. Stanley of San Quentin Prison, I was able to secure bits of muscle from the stomach and intestine of a healthy, strongly built man, aged thirty-five, whose abdomen was opened within twenty minutes after death by hanging. The stomach was full of food in the process of being digested. Except for some adhesions between duodenum and gall bladder, nothing abnormal was found in the abdomen. The small intestine, measured quickly before it had time to relax, was 3 meters long. This is less than half as long as one would expect from the results of studies made in dissecting rooms, but it agrees well with measurements on the living made by Scheltema and van der Reis and Schembra. I discovered years ago that measurements of bowel length must be made in the living animal if they are to be of any value.

Small pieces of muscle 2 cm. long and 7 mm. wide were removed from many parts of the wall of the stomach, small intestine, and colon. I noted that just as in laboratory animals, so in man, the muscle along the lesser curvature of the stomach was firmly fastened to the mucous membrane, much as the skin of the palm of the hand is fastened to the underlying fascia. It could be removed fairly easily in the fundus and with great ease in the pars pylorica. At the pylorus itself the muscle was firmly attached to the mucous membrane and in the duodenal cap the two layers were so nearly inseparable that in my attempts at dissecting them apart I repeatedly broke into the lumen of the gut.

Just as in laboratory animals, so in man, the intestinal muscle becomes thicker in the lower ileum as one approaches the ileocolic sphincter. It is thick also in the pelvic colon and rectum. In the upper part of the bowel the muscle, when cut, tended to pull apart but this was not true in the lower part of the colon where the tissue seemed to be rigid. It was easier to separate muscle from mucous membrane in the cecum and ascending colon than in the descending colon. The descending colon was found to be a hard, narrow tube as it so often is in nervous persons during life.

The strips, all cut longitudinally, were strung in known order on safety pins and placed in cold (6° to 13°C.) Locke's solution in a thermos bottle. Eight hours elapsed before work could be begun in the laboratory.

Latent periods in the stomach. The first studies were made on the latent periods of the strips from the stomach. (A short note on this phase of the work was published by the author in 1917.) One after the other the bits of muscle were placed in a warm moist chamber, suspended between two tiny wire serrefines, one of which was fastened by means of a fine copper wire to a light lever. A faradic tetanizing current obtained from a Porter

inductorium was led to the muscle by way of the serrefines. The figures in table 1 show that just as in laboratory animals, so in this man, the segment from the lesser curvature next to the cardia had the greatest irritability and the shortest latent period. It responded to the current when, with one dry cell in the primary circuit, the secondary coil was at 8 cm. As in the

TABLE 1

*Latent periods in different parts of the digestive tract of an executed criminal**

Stomach

DISTANCE TO SECONDARY COIL	NEAR CARDIA, LESSER CURVATURE	PREANTRUM, LESSER CURVATURE	PARS PYLORICA, LESSER CURVATURE	FUNDUS	MIDDLE, GREATER CURVATURE	PREANTRUM, GREATER CURVATURE
cm.						
8	3.00					
6	0.44 or 0.63	0.68 or 0.60	0.70 or 0.85	0.45	0.55	0.85
4	**0.19**	**0.27**	0.28 or 0.47	0.22 or 0.32	**0.32**	**0.26**

Small intestine

DISTANCE TO SECONDARY COIL	DUODENAL CAP	DUODENUM	DUODENO-JEJUNAL JUNCTURE	JEJUNUM 40 CM. BELOW THE LIGAMENT OF TREITZ	LOWER ILEUM	TERMINAL ILEUM
cm.						
6				1.5; 1.0		**0.40**
4	1.15?	0.24	0.26 or less	0.28 or 0.14?	0.28	**0.17**

Colon

DISTANCE TO SECONDARY COIL	CECUM	ASCENDING COLON AFTER 24 HOURS	TRANSVERSE COLON LEFT SIDE	SPLENIC FLEXURE	PELVIC COLON	RECTUM
cm.						
6	**0.45**	0.50			**0.81**	
4	**0.19**	**0.28**	0.60 or less	**0.52**	**0.20**	1.70
2						0.26?

* Faradic tetanizing current was used. Temperature 37° to 38°C. Bold face type indicates that results were unusually clear-cut.

lower animals studied, so in this man, there was a gradient in latent period from 0.19 second at the cardia to 0.28 or 0.47 second in the pars pylorica.

Unfortunately, the measurements made on some of the strips are doubtful because the lever rose so slowly from the base line. In others it rose so quickly that there could be no doubt about the length of the latent period. Some may ask: But why were not several tests made so that results could be averaged? The difficulty is that when studying intestinal

muscle, weak currents cannot be used because the responses are so variable, and strong currents sometimes injure the tissue so that the first response is the quickest and the only reliable one. With successive contractions the muscle may fail to relax and it may become fatigued or unresponsive. Furthermore, in comparing latent periods in many parts of the digestive tract it is necessary to work rapidly so that some strips will not have been kept outside the body and under adverse conditions much longer than others.

Latent periods in the small bowel. Table 1 shows the latent periods found in different parts of the small bowel. With the secondary coil at 6 cm., the only two strips to respond were those from the upper part of the jejunum and the terminal ileum just orad to the sphincter. As in dogs, so in this man, the duodenal cap had a long latent period. This may be due, at least in part, to its greater sensitiveness to the trauma of excisior and the injury wrought by deprivation of oxygen, but it may well be that the result obtained is comparable with those secured in other parts of the bowel, and that this sluggishness of the cap is real and just what one should expect to find in a segment which so commonly fails to empty itself of food.

In view of the uncertainties in the measurements, all that can be said is that in this man, as in animals, there appeared to be a slight gradation from short latent periods in the second portion of the duodenum to long latent periods in the lower ileum. Near the ileocecal sphincter the latent period again was short. This is probably an important detail and there are many reasons for believing that a highly efficient muscle is needed at this point to help the ileocecal sphincter in holding back the progress of material coming down the bowel. It is interesting to note that the actual figures are practically the same as those obtained in dogs and rabbits.

Latent periods in the colon. With the secondary coil at 6 cm., the only strips to respond were those from the cecum, ascending colon, and pelvic colon. The quickest and best contraction was obtained with muscle from the cecum. Again, with the coil at 4 cm., there were good quick responses with muscle from the cecum and pelvic colon. In view of the fact that the colon is a more sluggish organ than the small bowel it was surprising to find such short latent periods. It was not surprising to find a quick response about the sigmoid flexure because it has long been suspected that this is a region of high irritability. The muscle from the ascending colon was not tested until twenty-four hours after death but its reactions were then so good that its latent periods have been included in table 1.

Shape of the curves of contraction. With striated muscle from different parts of the body there is often a close relation between the length of the latent period, the steepness of the rise of the contraction curve, the total length of this curve, and the type of work that has to be done but this rule does not seem to apply to the contractile tissue of the digestive tract. In

the stomachs of the mammals studied the muscle at the cardia, which had the shortest latent period, was slow to relax, while that in the pars pylorica, which had the longest latent period, relaxed promptly.

In the case of the criminal studied, the contraction curves of muscle from different parts of the stomach were like those in animals, and the strip from the pars pylorica was the only one to relax promptly. Nothing definite can be said about the bowel except that there was a tendency to more rapid rise and fall in the curves made by muscle from the duodenum than in those made by muscle from jejunum and ileum. In the colon the shortest periods of rise and fall were in the curves made from muscle from the descending and pelvic segments.

Differences in tone. When strips of muscle were being removed from the stomachs of animals it was noticed that in the pars pylorica, and along the lesser curvature, the strip contracted so that it was smaller than the hole whence it had been removed, whereas in the fundus and in the greater curvature it often stretched so as to become larger than the hole. Later, when the strips were placed in a bath of warm oxygenated Locke's solution, those that had contracted most had to relax most before they could beat rhythmically. It was interesting therefore to note that in warm Locke's solution the strips from the human stomach all lost tone; the one from the lesser curvature near the cardia most, and after it, in order, the one from the preantral region, the one from the middle of the greater curvature, the one from the preantrum on the lesser curvature, the one from the fundus, and the one from the pars pylorica.

Rhythmic contractions of the stomach on the first day. The only muscle to show rhythmic activity on the first day was that from the cardia. This agrees with experiments on the stomachs of animals which showed that of all the various regions, that on the lesser curvature near the cardia has the greatest tendency to contract rhythmically.

Rhythmic contractions of the stomach after twenty-four hours. At eleven o'clock in the evening of the first day the strips were put in the ice box at 6°C. and were left until the following morning when again they were transferred to warm aërated Locke's solution and fastened to light levers. As in animals, after the imposition of adverse conditions, so here, the strip removed from the pars pylorica showed the first signs of recovery. The waves in all the records were irregular in rhythm and amplitude; the rates were slow, and no gradient in rate of contraction was observed. The only record closely resembling that commonly obtained in animals was the one from the pars pylorica. It showed the typical "church-steeples" rising every twenty seconds from a constant base line.

Rhythmic contractions in the small bowel. On placing the strips in warm Locke's solution there was a marked drop in tone of the muscle from the lower ileum. This drop was slight in the strip from the first portion of the

jejunum and absent in that from the duodenum. As in animals, so in man, the amplitude of contraction was greatest in the records from the strips of ileum; it was less in the jejunum and least in the duodenum.

The rates of rhythmic contraction were so irregular that little can be said about them. The second portion of the duodenum contracted fairly regularly at first with a rate of ten times a minute. Much of the time the ileum contracted about six times a minute but often there were series of small, more frequent contractions. It is suggestive that these rates agree with those observed in men and women with the roentgenoscope.

A little atropin added to the solution produced no definite effect, but a little epinephrin produced the typical relaxation seen in animals. Just as in the rabbit, so here, the effect was more transitory in the duodenum and jejunum than in the lower ileum. A little cocain had no effect. Magnesium sulphate produced the expected inhibition of the movement.

TABLE 2

*Latent periods of muscle from a carcinomatous stomach**

DISTANCE TO SECONDARY COIL	PREANTRAL REGION, GREATER CURVATURE	PARS PYLORICA, GREATER CURVATURE
cm.		
6	0.54	1.70
4	0.24; 0.27	1.74?; **0.55**
0	**0.22**; 0.19?	**0.27**

* A tetanizing faradic current was used. Bold face type indicates that results were unusually clear-cut.

Rhythmic contractions in the colon on the third day. On the third day after death, the strips of colonic muscle were put into warm oxygenated Locke's solution and shortly afterward the one from the rectum began to contract regularly, eight times a minute. Later those from the cecum and splenic flexure became active. A little barium chloride added to the solution caused a definite rise in tone while magnesium sulphate caused the usual marked drop. Epinephrin and atropin in the concentrations usually effective in animals produced no result.

STUDIES ON A CARCINOMATOUS STOMACH. Strips of muscle were removed from the greater curvature of the stomach of a woman who died from a carcinoma on the lesser curvature near the pylorus. In spite of the fact that this stomach was diseased, the latent periods (table 2) obtained were about the same as those observed in the executed criminal.

STUDIES ON THE STOMACH AND BOWEL OF A MAN WHO DIED OF NEPHRITIS. Strips of muscle were secured from various parts of the stomach and bowel of a man on whom necropsy was performed within thirty minutes after death from nephritis. As reported briefly elsewhere (Alvarez, 1916), strips

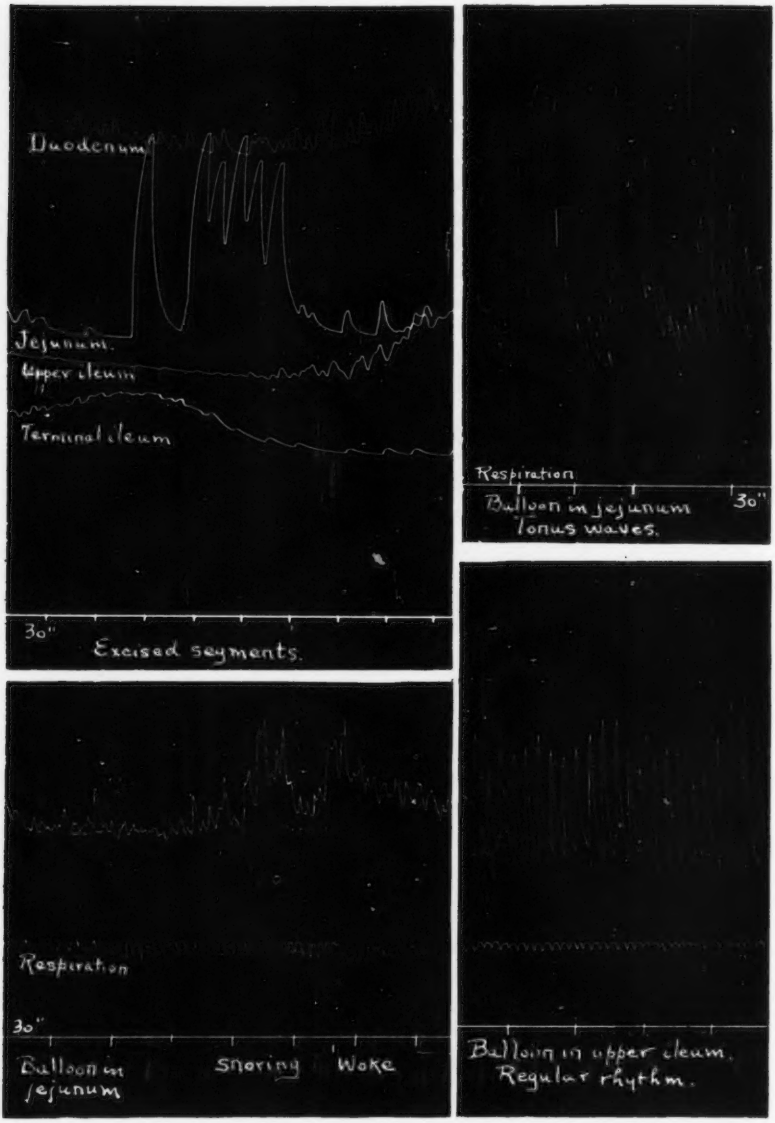

Fig. 1. Rhythmic contraction in the small bowel of man

from the stomach of this man, when placed in warm aërated Locke's solution, behaved almost exactly as do those from laboratory animals. The segment from the lesser curvature near the cardia showed the greatest rhythmicity and the fastest rate; the amplitude was small and the rhythm perfectly regular. The strip from the pars pylorica showed typical "church-steeples."

In figure 1 it will be seen that the strips from the small bowel contracted irregularly. As in animals, so here, the strip from the duodenum revealed the highest rhythmicity by beginning to contract first, while that from the terminal ileum was the last to begin. Table 3 shows that the rates were irregular and that there was no gradient from duodenum to ileum such as is seen in healthy rabbits, cats, and dogs. It must be remembered, however, that this man was anything but healthy, and that in some sickly animals there is no rhythmic gradient.

TABLE 3

*Rates of contraction in successive intervals of strips of muscle from the gastro-intestinal tract of a man who died of nephritis**

	BEATS EACH MINUTE					
Duodenum	13	10	7	6	8	7.5
Jejunum	6.5	5	5			
Upper part of ileum	7.5	18	12	8.7	9	
Terminal part of ileum	8	8.7	11	11		

* Temperature, 36.5°C.

STUDIES OF OTHER EXCISED SEGMENTS. A piece of jejunum removed at operation was placed in warm, aërated Locke's solution where it promptly began to contract rhythmically with a very low amplitude and a rate of from five to eight waves a minute. The next day after it had been in the ice box all night, it contracted with better amplitude and more regular rhythm. The rate was unchanged except when the lever was weighted; then sometimes it rose to sixteen a minute, the usual rate in the rabbit. Twenty-four hours later the muscle still contracted well, better in fact than on the second day. The rate was from four to seven a minute. Seventy-two hours after removal from the body, the muscle still contracted better than it did on the first day. The rate was from four to seven a minute. Five days after removal from the body the muscle still contracted regularly with a small amplitude and a rate of from five to eleven a minute.

Bits of jejunum and ileum removed from a man an hour and forty minutes after death from tuberculous meningitis were kept in the ice box for forty-eight hours, and then were placed in warm aërated Locke's solution. They soon contracted rhythmically but with a poor amplitude.

The rate of the jejunal muscle was from four to twelve a minute and that of the ileal muscle from four to eight a minute.

A piece of ileum was removed during partial colectomy for obstructing adhesions following extra-uterine pregnancy. Four and one-half hours later, strips of circular and longitudinal muscle were placed in warm aërated Locke's solution and after twenty-six minutes the longitudinal strip began to contract regularly with a small amplitude and a rate varying from eight to seventeen a minute. The mean of twenty measurements was twelve. In another half-hour the circular muscle began to contract slowly and irregularly. Forty-eight hours later the circular muscle contracted well with a rate varying from seven to nine a minute but the longitudinal muscle no longer showed much sign of life.

EXPERIMENTS WITH A JEJUNAL FISTULA. In 1914, I spent some time studying the intestinal movements of a man with a fistula into the first portion of the jejunum. This fistula was made with the hope of curing an ulcer situated on the lesser curvature of the stomach near the cardia. The man, about fifty years of age, was considerably emaciated at first, but with jejunal feeding he soon gained in weight and strength and the ulcer healed.

A small balloon was passed into the bowel and in records secured with it the rate of contraction was found to vary considerably from time to time. It was commonly between ten and twelve a minute, but at times, and especially when food was being digested, it rose so that it was from sixteen to twenty-four a minute. Especially during active digestion, there were tonus waves every minute, and sometimes these waves were the most striking feature of the record. When the balloon was allowed to travel down into the lower ileum, 200 cm. from the fistula, the rate of rhythmic contraction was found to be between seven and sixteen a minute. There was a suggestion of a gradient in rate of rhythmic contraction from jejunum to ileum but I could not be sure of it on account of the marked variations from moment to moment in all parts of the gut. In this respect the human bowel is like that of the dog and the cat and is not like that of the rabbit or the white rat. Possibly a gradient in rate is present during active digestion and absent at other times. In the jejunum the mean of twenty-five measurements was fourteen a minute and in the ileum, 165 cm. caudad, the mean of eighteen measurements was ten a minute.

The man slept much of the time when the balloon was in the bowel and so far as I could see sleep made little difference in the activity of the bowel. This agrees with the observations of Hines and Mead. Once, shortly before he woke but while he was still snoring, there was a marked rise in tone and activity.

Just as in Hess' experiment on dogs the downward pull on the balloon was marked in the jejunum and slight in the ileum. When I did not allow the balloon to move, the man sometimes complained of pain. Once when

the balloon was far down in the ileum I distended it more than usual and he complained of pain at one spot in the lower left quadrant. Relief came the moment the balloon was deflated. At one time when tonus waves were unusually deep he complained of "gas pain" but there was no time relation between the tonus waves appearing in the record and exacerbations of the pain.

Psychic tone. In another article soon to be published I have described a number of observations made on this man and on the other patients studied that strongly suggested that with the sight, thought, or tasting of food there goes an increase in the amplitude of the contractions.

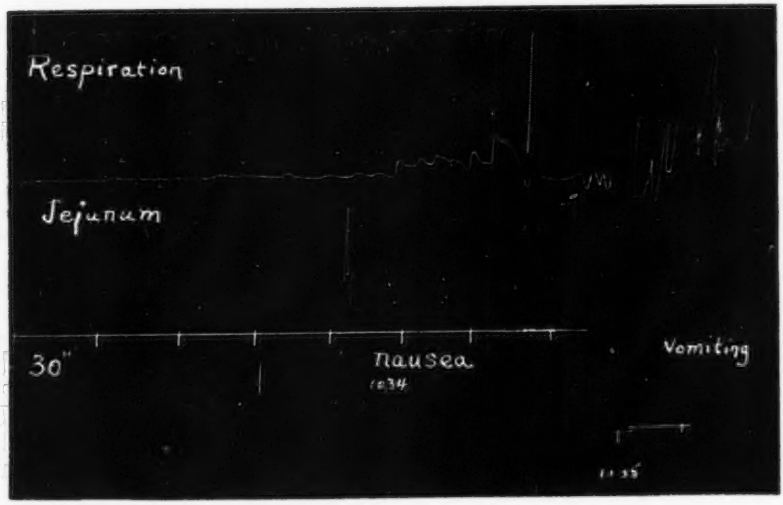

Fig. 2. Record of activity in the jejunum preceding vomiting. The upper record represents respiration. Unfortunately the disturbance came just as the writing point reached the end of the drum.

Vomiting. It is interesting to note that in this man, and also in another with a jejunal fistula, the egg-milk mixture given through the tube was sometimes regurgitated or vomited. One day, while I was recording the contractions of the jejunum, there was a rise in tone and an increase in the amplitude of the contractions and two minutes later the man vomited (fig. 2).

Difficulties in feeding. Pain was produced when the man was given food by the mouth and by the fistula at the same time. Apparently the putting of food into the jejunum raised its tone and interfered with the progress of material coming down from above. Cleansing enemas, if injected about

mealtime, also produced much distress in the small bowel and greatly increased the amount of food regurgitated through the fistula.

It was found so essential to inject the food slowly and at body temperature that it was generally given as a Murphy drip. When cold food was given rapidly the patient was much distressed with colicky pains similar to those experienced by some persons with a large gastro-enterostomy opening or with a relaxed, inefficient pylorus which allows the stomach to pour its contents too quickly into the bowel.

Another curious observation was that when the balloon was distended in the jejunum the man often dropped into a sound sleep. Ivy has told me that he has observed the same thing in dogs and Pearcy and Allen noticed it when they put balloons into the stomachs of men. It suggests that the drowsiness that many persons experience after meals is not due to the absorption of end-products of digestion but to the soothing effect of rhythmic contractions.

Observations on another man with jejunostomy. Studies similar to those just described were carried out on a man with carcinoma of the cardia who two months previously had had an opening made into the jejunum. The rhythmic contractions in this man appeared in groups of from three to thirty, separated by quiet intervals, and the rate varied from eight to twenty-three a minute. As in the other men and women studied, the rate varied from moment to moment.

Studies on the colon in a case of rectal carcinoma. An attempt to record the movements of the large bowel in a woman with a carcinoma of the rectum and a permanent colostomy on the left side were not successful because the bowel was so unresponsive. When the recording balloon was inserted and distended the bowel responded with one or more large contractions, usually with smaller ones superimposed, and then it became refractory. Some of the sluggishness may have been due to the carcinoma but experience with animals makes me think that much or all of it was characteristic for this part of the bowel.

Study in a case of ventral hernia. In 1917 I saw a woman about fifty-five years of age with a large ventral hernia. The bowel was covered only by peritoneum and skin and the contractions could easily be seen. The segmenting to and fro movements closely resembled those of animals, and from time to time there were shallow rush waves. Some powerful cramp-like contractions were seen at times; contractions that looked as if they ought to produce distress but there was none. Lifting up a loop of bowel between the fingers seemed for a moment to inhibit its activity but pinching had no effect. The application of hot water bottles and ice bags had no visible effect on the amplitude or the rate of the contractions.

When I injected into a vein 0.5 cc. of a 1:1,000 solution of epinephrin the bowel immediately became quiet and remained so for three minutes.

Then there appeared peristaltic rushes and violent to and fro movements which lasted for five minutes. Associated with the rushes was a feeling that the bowels were going to move.

Psychic effects. One day when for some time the bowel had been quiet, the nurse appeared with luncheon, and within a few seconds, a peristaltic rush appeared and the rhythmic movements became much more active. Shortly after this, as the patient ate the meal, there were several more rushes. Similarly, in another patient with a fistula in the jejunum the taking of food always started a series of rushes which caused material to bubble out of the opening. When mealtime was approaching I tried to stimulate the bowel by talking about food but saw no sign of a psychic increase in activity. This may have been due to the fact that the patient had no appetite.

With the help of small cups such as are used for making tracings of pulsations in the neck I obtained graphic records of the rhythmic movements and found that the rate in a loop of what was probably ileum varied from four to nine a minute with the mode about eight.

THE CAUSE OF BELCHING. A small woman with a very thin abdominal wall came to the clinic with obstruction at the outlet of the stomach, marked distention of that organ, and easily visible peristaltic waves sweeping normally to the pylorus. These waves were watched for considerable periods of time until finally one was seen running orad. About the time this must have reached the cardia the patient belched. The observation interested me much as I have long been almost certain that belching and many of the distressing sensations in cases of indigestion are due to reverse waves or ripples which break against the cardia.

SUMMARY

Rates of rhythmic contraction were counted with the roentgenoscope and found to be about ten a minute in duodenum, jejunum, and ileum.

Strips of muscle were removed from the stomach and bowel of an executed criminal. In some places the muscle was easy to remove, in others it was firmly attached to the mucosa. The small intestine was three meters long.

As in animals, so in man, there was a gradation in latent period in the stomach from short intervals at the cardia to long ones in the pars pylorica. There was a suggestion of a similar gradient from duodenum to lower ileum. The muscle of the duodenal cap was very sluggish while that of the terminal ileum next to the sphincter was very active.

The latent periods in different parts of the digestive tract were the same as those found in laboratory animals. In the colon the shortest latent periods were obtained in the cecum and sigmoid flexure. The shapes of the contraction curves were different in different parts of the tract. The muscle contracted rhythmically three days after excision.

The records of rhythmic activity of muscle from various parts of the stomach of man closely resembled those of muscle of similar origin in laboratory animals, with a fast rate at the cardia and a slow one in the pars pylorica. Rhythmic contractions of muscle from the bowel were irregular in rate and amplitude. The rate varied markedly from moment to moment, and there was no definite gradient from duodenum to ileum. There were marked tonus waves.

In one man a bit of jejunum removed at operation contracted rhythmically on the third day better than on the first, and it was still able to contract after five days in the ice box.

In two men, records were obtained by passing a balloon into the bowel though a jejunal fistula. The rate of rhythmic contraction was variable but there was a suggestion of a gradient from duodenum to ileum. The rates were faster during digestion. There was no depression of activity during sleep. The pull on the balloon was most marked in the jejunum and least in the lower ileum.

In a number of subjects studied there was evidence of a psychic tone produced by the thought or sight or taste of food.

Food introduced into the fistula was often regurgitated and occasionally vomited. A rise in tone and activity of the jejunum preceded vomiting. The giving of food by mouth and fistula at the same time caused pain or distress. The jejunum tolerated food only when it was given slowly and at body temperature.

The presence of the balloon in the bowel seemed to induce sleep.

The introduction of the balloon into the colon showed that this region of the bowel is comparatively unresponsive to distention.

Rush waves and rhythmic contractions, similar to those in animals, were seen in a woman with a large ventral hernia. Heat and cold applied to the abdomen had no effect on these movements. Epinephrin temporarily inhibited them but after three minutes caused them to be very active; rushes appeared and there was a desire for bowel movement.

In a woman with gastric peristalsis easily visible through a thin abdominal wall a reverse wave ended in an eructation.

BIBLIOGRAPHY

Alvarez, W. C. 1916. This Journal, xl, 585.
1917. This Journal, xlii, 435.
Ganter, G. 1923. Pflüger's Arch., cci, 101.
Hess, J. 1886. Deutsch. Arch. f. klin. Med., xl, 93.
Hines, L. E. and H. C. A. Mead. 1926. Arch. Int. Med., xxxviii, 536.
Pearcy, J. F. and T. D. Allen. 1927. This Journal, lxxxii, 56.
Plant, O. H. and G. H. Miller. 1926. Journ. Pharm. Exper. Therap., xxvii, 361.
Reis, van der and F. W. Schembra. 1924. Zeitschr. f. d. ges. exper. Med., xliii, 94.
Scheltema, G. 1908. Zeitschr. f. klin. Med., xlv, 505.
Weitz, W. and W. Vollers. 1926. Zeitschr. f. d. ges. Exper. Med., lii, 723.

STUDIES IN THE METABOLISM OF MUSCLE

II. The Respiratory Quotient of Exercising Muscle[1]

HAROLD E. HIMWICH and MILTON I. ROSE

From the Department of Physiology, Yale University, New Haven

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The present report, like the previous one of this series (Himwich and Castle, 1927), is a study of the foodstuffs supplying energy for mammalian muscle. In the previous paper it was shown that "resting muscle *in situ* has a respiratory quotient not necessarily of unity but probably close to that of the whole body." This means that "resting muscle under normal conditions oxidizes besides carbohydrate either fat or protein, or both, probably in the same proportions as does the rest of the body."

It is even of greater interest to determine the source of energy for muscular exercise. Does the character of the oxidative processes in the muscles change so that carbohydrates only can be oxidized, or may non-carbohydrate foodstuffs also supply energy for work just as they do under resting conditions? The data in the literature appear to find support for both viewpoints. Meyerhof (1920) observed a respiratory quotient of unity in frog's muscle which had been removed from the body and then caused to exercise. In A. V. Hill's laboratory, the whole organism was studied, human beings serving as the experimental animals (Furusawa, Hill, Long, Lupton, 1924). It was found that early in exercise the respiratory quotient was 1.0 and then gradually fell. These data were interpreted as meaning that the fuel of muscle was carbohydrate since a short bout of exercise was conducted simply at the expense of carbohydrate, but if exercise was prolonged metabolic processes were stimulated which restored some of the carbohydrate at the expense of fat. At this point it is not necessary to consider the problem of the formation of carbohydrate from fat. However, other data do not confirm the observation that the respiratory quotient is raised to unity during exercise. Chauveau (1896), Zuntz (1911), Anderson and Lusk (1917), Benedict and Cathcart (1918), Krogh and Lindhard (1920), Richardson and Levine (1925), Henderson and Haggard

[1] The experimental data in this paper are taken from the dissertation submitted by M. I. Rose in partial fulfillment of the requirement for the degree of Doctor of Philosophy, Yale University, 1927.

Preliminary report. H. E. Himwich and M. I. Rose, Proc. Soc. Exp. Biol. and Med., 1926, xxiv, 169.

(1925), Wilson, Levine, Rivkin and Berliner (1927), Rapport and Ralli (1928), and more recently Marsh (1928) obtained quotients during exercise which were not very different from those found during rest. From these data it is quite possible that in addition to carbohydrates, non-carbohydrates may also be utilized. In order to attack this problem at its very site, the muscles while still functionally part of the body have been studied by analyzing samples of blood going to and coming from them. Thus, the respiratory quotients of the working muscles were determined directly without the mediation of the expired air and therefore uncomplicated by the gaseous exchange of other parts of the body.

METHOD. The methods are essentially those described in the previous paper (Himwich and Castle, 1927). In order to compare the respiratory quotient of exercising muscle as determined from gaseous exchange in the blood with the respiratory quotient of the whole animal before its muscles were stimulated to activity as well as during exercise, it was necessary to have a preparation which would remain in the same position during rest and exercise. This was obtained by Schmidt's (1923) method of decerebration of the dogs which were used as the experimental animals throughout the study. To obtain the respiratory quotient of the entire animal, the expired air of the decerebrate dogs was collected through a tracheotomy tube in a spirometer from which air samples were removed for analyses for carbon dioxide and oxygen by the Haldane-Henderson apparatus. In a similar manner, expired air was collected during exercise. The respiratory quotient of the exercising muscle was determined with the aid of two types of muscle preparation, each stimulated to activity by means of a tetanizing current. In the first type of preparation the femoral vessels were exposed with otherwise intact lower extremities. The second type of muscle preparation consisted of the gastrocnemius and flexor digitorum sublimis muscles with blood supplies isolated from the remaining muscles of the leg. However, unlike the previous work (Himwich and Castle, 1927) the femur was not notched nor was its marrow cavity packed. Samples of arterial and venous blood were drawn simultaneously from the femoral vessels during a period of exercise which was begun from 2 to 15 minutes previous to the collection of the blood. The blood samples were taken directly from the artery and vein except in the experiments on the isolated muscles where the venous blood was drawn through a cannula and a calibrated glass tube as described earlier (Himwich and Castle, 1927). With the isolated muscle preparation it was thus possible to measure the blood flow and, therefore, calculate oxygen consumption. Twenty cubic centimeters of blood were collected under oil and kept air-tight in glass containers over mercury. To prevent coagulation and glycolysis the containers were provided with sufficient sodium oxalate and potassium fluoride in dry powdered form to make a 0.2 per cent and 0.1 per cent solu-

tion respectively with 20 cc. of blood. The solutions of sodium oxalate and potassium fluoride had previously been adjusted to pH 7.3. The containers were kept in an icebox except during the removal of the 1 cc. samples for analysis. One portion of the blood was analyzed directly for carbon dioxide and oxygen. On a second portion the carbon dioxide and oxygen capacities were determined. An improvement in the method of saturation of the blood samples for this determination was instituted. The gas mixture of 5½ per cent carbon dioxide in oxygen was passed through water heated to the temperature of the water bath (38°), before entering the tonometer. Since the gases were saturated with water vapor they could cause no change in the concentration of the blood in the tonometer. All blood samples were analyzed by the method of Van Slyke and Neill (1924) and checks were obtained to 0.2 volume per cent.

RESULTS. Forty-nine experiments were performed on thirty dogs. The experiments fall into two major groups; one on dogs fasted from 5 to 15 days, and another on dogs fed on a diet of ordinary dog biscuit which is made of flour, corn meal, meat and bran. Each of these groups may again be divided into two minor groups; some in which the venous blood was drawn from the femoral vein, carrying the return of the whole lower extremity, and others where the venous blood was drawn after the circulation of the gastrocnemius muscle and flexor digitorum sublimis had been isolated. For the purpose of discussion the groups of experiments may be divided into four series as follows:

Fasted dogs:	
Whole lower extremity	Series 1
Isolated muscle	Series 2
Fed dogs:	
Whole lower extremity	Series 3
Isolated muscle	Series 4

Whenever possible observations on two exercise periods were made on a single dog. The second period followed the first by approximately 1 hour. Thus the tables which are presented indicate a single respiratory quotient on certain days and two on others.

The differences between the oxygen and carbon dioxide capacities and contents of arterial and venous blood as well as those between the respiratory quotients from the blood and air are not presented in tables 1, 2, 3 and 4.

Series 1—Whole lower extremity—Fasted dogs. From column 3 of table 1 it may be seen that of the 14 values for oxygen capacity obtained from arterial and venous blood in all but one of the observations the oxygen capacities of the arterial and venous blood are close. In 9 of 14 observations the oxygen capacity of the venous blood is greater than that of the arterial, indicating an increased concentration in the venous blood. How-

ever, in one observation only, that of July 28th, is the difference beyond the analytical error, which is 0.4 volume per cent. The carbon dioxide capacity values (column 4) reveal the fact that in 10 of 14 cases there is a relative decrease in the venous blood and in 6 of these 10 fall is the significant.

TABLE I

Whole lower extremity—fasted dogs

Oxygen and carbon dioxide capacities and contents of arterial and venous blood and respiratory quotients of blood and air

1	2	3		4		5		6		7	8	9
DATE	DURATION OF PRELIMINARY EXERCISE	O_2 CAPACITY		CO_2 CAPACITY		O_2 CONTENT*		CO_2 CONTENT		R.Q. BLOOD IN EXERCISE	R.Q. EXPIRED AIR AT REST	R.Q. EXPIRED AIR IN EXERCISE
		Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous			
	minutes											
July 28	11	26.36	27.20	34.47	33.66	26.77	6.33	25.54	42.26	0.81	0.85	0.87
July 29	16	27.20	27.02	31.42	31.77	26.60	18.33	23.89	30.31	0.78	0.81	0.88
July 29	16	27.32	27.58	30.41	30.38	26.78	6.61	23.71	39.78	0.80		0.79
Aug. 1	11	27.14	27.31	32.95	31.60	22.31	4.47	26.07	44.27	0.82	0.83	0.90
Aug. 1	11	27.62	27.90	28.83	26.14	25.83	2.48	28.10	48.36	0.87		0.83
Aug. 6	16	26.71	27.03	31.07	29.14	25.15	2.36	15.25	36.86	0.94	0.85	0.87
Aug. 6	14	28.49	28.89	22.16	22.54	24.87	4.69	11.97	27.54	0.77		0.87
Aug. 11	16					23.39	6.73	18.21	32.37	0.85	0.80	0.84
Aug. 11	16	21.85	21.72	28.47	28.23	21.08	3.05	13.96	27.99	0.78		0.85
Aug. 20	16	29.66	29.34	37.01	36.29	21.33	6.72	36.69	46.50	0.88	(1.04)	(1.28)
Aug. 20	11					27.71	6.21	36.81	54.78	0.84		(2.+)
Aug. 23	12	26.99	26.95	34.13	34.22	24.71	7.47	35.06	48.62	0.79	0.76	0.76
Aug. 26	11	18.73	18.75	35.45	35.41	18.01	7.70	27.84	35.78	0.77	0.77	0.70
Aug. 27	7	23.16	23.40	26.65	26.24	23.10	6.03	19.67	31.51	0.69	(1.32)	0.88
Aug. 30	11	24.91	24.84	33.89	34.01	22.37	11.88	37.39	44.42	0.67	0.80	0.75
Aug. 30	5	23.53	23.71	38.33	36.62	23.11	11.15	40.56	50.62	0.84		0.77
Average										0.80 ±0.05	0.81 ±0.03	0.83 ±0.05

* Contents include dissolved oxygen in addition to oxygen in combination with hemoglobin.

In columns 5 and 6 are to be found the oxygen and carbon dioxide contents of arterial and venous blood from which the respiratory quotients are calculated. All of the 16 quotients are within the physiologic range and the average for all is 0.80 with a mean deviation of ±0.05 for a single determination (column 7).

We see in column 8 that the average respiratory quotient from the air of the resting animal is 0.81 with a deviation of ±0.03 for a single deter-

mination. This value is not significantly different from the mean respiratory quotients yielded by the air and by the blood during the exercise of the lower extremities. The average differences (not tabulated) between the blood respiratory quotients and the air respiratory quotients during rest and exercise are 0.04 and 0.07 respectively.

TABLE 2

Isolated muscle—fasted dogs

Oxygen and carbon dioxide capacities and contents of arterial and venous blood and respiratory quotients of blood and air

1	2	3		4		5		6		7	8	9
DATE	DURATION OF PRELIMINARY EXERCISE	O_2 CAPACITY		CO_2 CAPACITY		O_2 CONTENT*		CO_2 CONTENT		R.Q. BLOOD IN EXERCISE	R.Q. EXPIRED AIR AT REST	R.Q. EXPIRED AIR IN EXERCISE
		Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous			
	minutes											
Oct. 18	11	31.03	31.34	35.69	32.55	30.76	13.71	20.58	35.38	0.87	0.81	0.90
Oct. 18	4	30.63	30.65	34.38	34.31	29.52	14.76	24.67	37.05	0.84		
Oct. 25	10	23.34	23.66	30.59	30.57	24.78	4.20	20.30	37.65	0.84	0.86	0.98
Nov. 1	14	26.17	26.62	24.45	23.02	26.03	4.93	12.84	30.44	0.83	0.80	(1.03)
Nov. 22	12	21.55	21.48	35.97	34.60	21.60	13.93	29.90	34.83	0.64	0.82	0.89
Nov. 26	9	21.90	21.46	35.55	29.50	21.66	8.14	25.53	34.49	0.66	0.75	0.88
Nov. 26	5	23.34	23.54	30.91	31.87	21.87	4.69	17.30	31.17	0.81		0.95
Dec. 3	8	19.03	18.79	32.31	30.18	18.47	6.89	25.65	34.27	0.74	0.82	(1.15)
Dec. 3	6	18.15	18.14	25.17	24.64	14.97	4.11	24.18	32.23	0.73		(1.03)
Dec. 13	10	27.61	27.62	31.99	30.16	27.71	13.65	20.52	31.42	0.78	0.72	0.87
Dec. 13	7	27.26	26.25	27.55	29.63	26.98	8.71	12.78	27.73	0.82		0.88
Average										0.78 ±0.06	0.80 ±0.03	0.96 ±0.07
												0.91† ±0.03

* Contents include dissolved oxygen in addition to oxygen in combination with hemoglobin.

† Omitting respiratory quotients above unity.

Series 2—Isolated muscle—Fasted dogs. The oxygen capacities found in table 2, column 3, reveal differences that are not significant. The carbon dioxide capacities (column 4) are significantly affected in all but two cases, seven suffering a fall while the remaining two indicate a rise of this value in the venous blood.

From the series of gas analyses (columns 5 and 6) the average respiratory quotient of the blood representing 11 determinations on 7 dogs (column 7) is 0.78 ±0.06, a figure close to the average for the exercising

muscles of the whole lower extremity of fasted dogs in series 1 which is 0.80 ±0.05. With the exception of the respiratory quotient of 0.64 on November 22nd, and 0.66 on November 28th, all the respiratory quotients are within the physiologic range.

Column 8 shows that the average of the quotients obtained from the expired air of the resting animals, 0.80 ±0.03, is closely approximated by that of the respiratory quotients from the blood during exercise, 0.78 ±0.06, corroborating the results obtained in Series 1. The mean difference of 0.07 between individual respiratory quotients from the air at rest and the

TABLE 3

Whole lower extremity—fed dogs

Oxygen and carbon dioxide capacities and contents of arterial and venous blood and respiratory quotients of blood and air

1	2	3		4		5		6		7	8	9
DATE	DURATION OF PRELIMINARY EXERCISE	O_2 CAPACITY		CO_2 CAPACITY		O_2 CONTENT*		CO_2 CONTENT		R.Q. BLOOD IN EXERCISE	R.Q. EXPIRED AIR AT REST	R.Q. EXPIRED AIR IN EXERCISE
		Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous			
	minutes											
Jan. 14	9	22.61	22.53	36.86	36.85	22.25	8.41	33.09	45.43	0.89	0.88	0.90
Jan. 14	5	21.89	22.23	37.48	36.43	21.57	7.88	33.30	45.64	0.90		0.98
Jan. 21	8	23.68	24.01	36.86	35.89	22.36	7.65	28.61	43.98	1.04	0.87	0.95
Jan. 21	5	22.05	21.84	33.62	33.42	21.32	8.63	34.33	44.62	0.81		0.92
Jan. 28	10	30.55	30.53	35.02	35.41	29.71	14.02	28.36	42.64	0.91	0.93	0.96
Jan. 28	4	30.13	29.93	34.16	34.14	29.13	10.86	27.34	44.37	0.93		0.94
Mar. 2	4	24.16	24.48	33.60	33.58	24.16	5.12	24.50	42.46	0.94	0.90	0.94
Mar. 2	4	24.54	24.42	33.11	33.08	24.40	10.93	20.30	31.99	0.87		0.98
Average										0.91 ±0.04	0.90 ±0.02	0.95 ±0.02

* Contents include dissolved oxygen in addition to oxygen in combination with hemoglobin.

respiratory quotients from the blood obtained in exercise is somewhat greater than in series 1. The effect of exercise of the isolated muscles on the respiratory quotient of the whole body is indicated in column 9. Here we have an average of 0.96 ±0.07 for the 11 quotients listed, or 0.91 ±0.03 omitting the three respiratory quotients above unity.

Series 3—Whole lower extremity—Fed dogs. Column 3 presents the fact that in eight determinations of the oxygen capacity there is no significant difference between the concentrations of the blood in the artery and vein. The data also show (column 4) that in seven of the eight observations there is a relative decrease in the carbon dioxide capacity of the venous blood but in only two instances is this decrease beyond the experimental error.

In column 7 it may be seen that the eight respiratory quotients calculated from the blood data of 4 dogs fed on dog biscuit until the day of the experiment averaged 0.91 ±0.04 in contrast with the value 0.80 ±0.04 obtained from the blood of the whole exercising extremity of the fasted

TABLE 4

Isolated muscle—fed dogs

Oxygen and carbon dioxide capacities and contents of arterial and venous blood and respiratory quotients of blood and air

1	2	3		4		5		6		7	8	9
DATE	DURATION OF PRELIMINARY EXERCISE	O_2 CAPACITY		CO_2 CAPACITY		O_2 CONTENT*		CO_2 CONTENT		R.Q. BLOOD IN EXERCISE	R.Q. EXPIRED AIR AT REST	R.Q. EXPIRED AIR IN EXERCISE
		Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous			
	minutes											
Nov. 8	11	24.85	26.22	35.52	32.63	24.96	6.23	27.89	46.22	0.98		
Nov. 19	7	21.16	21.38	31.80	33.12	20.36	7.58	21.66	34.93	1.04	0.95	1.06
Dec. 6	12	28.05	28.09	33.71	31.77	25.96	10.86	27.30	41.32	0.93	0.94	0.88
Dec. 6	6	25.09	25.27	30.62	27.08	24.15	6.93	19.50	36.35	0.98		0.87
Jan. 3	7	22.64	22.59	37.92	33.85	22.45	10.23	33.22	47.36	(1.16)	0.92	(1.35)
Jan. 3	6	22.09	22.08	34.87	30.11	21.93	7.49	44.34	26.73	(1.22)		(1.30)
Jan. 10	7	19.58	19.93	36.28	35.70	19.51	13.17	30.69	34.92	(0.67)	0.95	0.88
Feb. 12	12	29.49	29.00	29.44	29.09	29.22	7.46	16.89	39.35	1.03	0.96	1.04
Feb. 18	8	26.63	26.70	31.22	30.31	26.80	22.50	19.09	23.53	1.03	0.96	1.03
Feb. 25	12	17.83	19.76	31.72	27.50	18.98	3.16	20.89	37.17	1.03	0.87	1.03
Feb. 25	12	18.36	17.55	31.52	23.46	20.22	2.23	12.93	32.41	1.08		1.05
Mar. 5	5	21.71	22.11	31.52	27.38	21.70	11.16	17.99	27.02	0.86	(1.05)	(1.13)
Mar. 5	4					21.30	8.76	13.92	24.59	0.85		(1.10)
Average										0.98 ±0.07	0.94 ±0.02	1.06 ±0.11
												0.98† ±0.07

* Contents include dissolved oxygen in addition to oxygen in combination with hemoglobin.

† Omitting respiratory quotients in brackets.

dogs in series 1. Of the eight respiratory quotients in series 3 only one is close to 0.80.

We note in column 8 that the average respiratory quotient obtained from the expired air of the dogs at rest, 0.90 ±0.02, and in exercise, 0.95 ±0.02, are in agreement with the mean respiratory quotients yielded by the blood during exercise, 0.91 ±0.04. The average differences between the respiratory quotients from the blood and the respiratory quotients

from the expired air of the dogs at rest and exercise are 0.04 and 0.07 respectively.

Series 4—Isolated muscle—Fed dogs. Examination of column 3 discloses the fact that in 8 of 12 observations there is a greater oxygen capacity and, therefore, an increased concentration of the venous blood which, in two instances, is greater than the experimental error. With the exception of the experiment on February 12th, when the carbon dioxide capacity

TABLE 5

Blood flow and oxygen consumption of exercising isolated muscle preparation

DATE	WEIGHT OF DOG	WEIGHT OF MUSCLE	DURATION OF PRELIMINARY EXERCISE	BLOOD FLOW PER SECOND PER GRAM OF EXERCISING MUSCLE	O_2 CONSUMPTION PER HOUR PER GRAM OF EXERCISING MUSCLE	O_2 CONSUMPTION PER HOUR PER GRAM OF ENTIRE ANIMAL AT REST
	kgm.	*gram*	*minutes*	*cc.*	*cc.*	
Oct. 18	24.0	90.0	6	0.0051	3.24	0.420
Oct. 25	21.0	87.4	18	0.0018	1.44	0.258
Nov. 1	19.0	79.0	14	0.0017	1.44	0.474
Nov. 8	20.0	85.0	15	0.0033	2.16	
Nov. 26	22.0	89.0	9	0.0020	1.08	0.786
Dec. 3	27.0	92.0	15	0.0099	3.96	
			12	0.0091	3.60	0.408
Dec. 6	20.5	89.0	10	0.0030	1.80	0.336
			4	0.0029	1.80	
Dec. 13	24.0	103.0	10	0.0046	2.16	0.360
			7	0.0029	1.80	
Jan. 3	19.5	67.0	3	0.0023	1.08	0.342
			3	0.0020	1.08	
Jan. 10	29.25	130.0	3	0.0059	1.44	0.270
Feb. 12	25.0	100.0	7	0.0037	2.88	0.774
Feb. 18	23.0	87.0	7	0.0104	1.80	0.564
			5	0.0013	1.08	
Mar. 5	19.5	73.0	3	0.0081	3.24	
			3	0.0014	5.04	
Averages				0.0048	2.22	0.454

remains unchanged, and on November 19th when it rises, the capacity falls, in most instances to a marked degree.

Column 7 contains the data concerning the respiratory quotients obtained from the blood in the last series of experiments. Three quotients have not been included in the average which is thus 0.98 ± 0.07.

The average of the respiratory quotients of the expired air of the resting animals (column 8) is 0.94. The mean respiratory quotient of the expired air obtained during exercise (column 9) is higher than the resting

one unless we exclude the four quotients in brackets when the mean is 0.98 ±0.07. A similar rise in the respiratory quotient was noted in the experiments on the isolated muscles of fasted dogs in table 2.

In the experiments here presented the muscles were continuously stimulated by a constant tetanizing current, beginning some time before and continuing throughout the taking of the samples. Since these experiments, further studies have been made on muscles which have been stimulated by a single induction shock once every second, thus allowing a longer time for recovery. In one such experiment, with blood taken during the second minute of exercise, the respiratory quotients both from the expired air and from the blood were the same, 0.78. This result agrees with the present findings where a tetanizing current was used, and indicates that no gross abnormality was introduced by this manner of stimulation.

Oxygen consumption. Table 5 summarizes the data on the oxygen consumption of 13 exercising isolated gastrocnemius preparations. In addition, the last column of the table contains the figures for the oxygen consumption per gram per hour of the whole body at rest as determined by analyses of expired air. The average oxygen consumption for working muscle per gram per hour is 2.22 cc., while that of the whole animal at rest is 0.454 cc. The average blood flow in the muscle during exercise is 0.0048 cc. per gram per second.

TABLE 6

Summary of results of the four series of experiments

SERIES	NUMBER OF EXPERIMENTS	NUTRITIVE CONDITION OF DOGS	PREPARATION	AIR R.Q. WHOLE ANIMAL REST	BLOOD R.Q. MUSCLE EXERCISE	AIR R.Q. WHOLE ANIMAL EXERCISE
1	16	Fasted	Lower extremity	0.81 ±0.03	0.81 ±0.05	0.83 ±0.05
2	11	Fasted	Isolated muscle	0.90 ±0.03	0.78 ±0.06	0.96 ±0.07
3	8	Fed	Lower extremity	0.90 ±0.02	0.91 ±0.04	0.95 ±0.02
4	14	Fed	Isolated muscle	0.94 ±0.02	0.98 ±0.07	1.06 ±0.10

DISCUSSION. Table 6 which summarizes the data shows that the respiratory quotient obtained from the blood of the exercising muscle usually agrees with that of the expired air of the resting animal. The evidence thus points to no exclusive carbohydrate utilization during exercise. The working muscles seem to oxidize foodstuffs in the same proportion as the whole organism during rest.

In series 1 and 3 the respiratory quotient obtained from the expired air during the exercise of the whole lower extremity is in fair agreement with the respiratory quotient of the resting animal, while in series 2 and 4 on the isolated muscle the respiratory quotient observed in the expired air during exercise is higher than the respiratory quotient at rest. It will

be noticed that it is those series which yield the higher respiratory quotient from the air during exercise which also show significant decreases in the carbon dioxide capacity in the venous blood. The amount of carbon dioxide released in the lungs is not dependent solely on the carbon dioxide produced by oxidations, but also on the extra carbon dioxide which may be expelled or retained by changes in the carbon dioxide capacity of the blood. Under resting conditions where there is no lowering of the carbon dioxide capacity of the mixed venous blood by a fixed acid, the amount of carbon dioxide released by such venous blood in passing through the lungs is not increased by extra carbon dioxide. Thus, in the experiments listed in tables 1 and 3 on the whole lower extremity, where in the majority of the observations there is an unchanged carbon dioxide capacity of the venous blood, the respiratory quotients of the working animals agree with those obtained from the animals at rest. During the time when the blood was being drawn no extra carbon dioxide was eliminated and if foodstuffs were still available in the same proportion as during rest, one might expect in the expired air collected during exercise respiratory quotients similar to those obtained in the resting air.

When conditions are such that exercise is accompanied by a fall in the carbon dioxide capacity of the venous blood, due to a release of lactic acid, some of the bicarbonate of the blood is broken up with the formation of basic lactate and carbonic acid. Carbon dioxide is carried in two forms, as carbonic acid and as bicarbonate. If some of the bicarbonate is decomposed it means that more of the carbon dioxide must be carried as carbonic acid with a consequent increase in the carbon dioxide tension of the blood. This causes extra carbon dioxide to be given off in the lungs as equilibrium with the alveolar carbon dioxide tension there becomes established. Thus the respiratory quotient obtained from the expired air is raised as in series 2 and 4. Examination of the individual quotients of the expired air during exercise and their corresponding carbon dioxide capacities, however, reveals no quantitative relationship between the height of the quotient and the changes in the carbon dioxide capacity, possibly because the values for carbon dioxide capacity are representative of the changes occurring in the exercising muscles only and not of the whole body and because we do not know that a constant alveolar carbon dioxide tension obtained.

The decrease of the carbon dioxide capacity of the venous blood, with the freeing of carbon dioxide, may cause an increased carbon dioxide tension in the blood and alveolar air unless the tension is decreased by an accompanying hyperpnea. However, even if the carbon dioxide tension was changed the respiratory quotient observed in the blood was not altered. Perhaps the carbon dioxide produced by oxidations continues to pour into the blood stream, possibly in the same amounts, the whole

process however occurring at a different tension. However, in some instances, notably in the two quotients 1.16 and 1.22 of January 3rd (table 4, column 7), the respiratory quotients obtained from the blood were higher than the average from the expired air during rest. Just as a fall in the carbon dioxide capacity of the blood may force extra carbon dioxide from blood to alveolar air, raising the respiratory quotient of the air, so in muscle the accumulation of lactic acid may force extra carbon dioxide from muscle to blood, raising the apparent respiratory quotient of the blood (Fenn, 1928). This unusual outpouring of extra carbon dioxide, unencountered in any other instance, produced a similar result in the lungs for the respiratory quotients from the air were 1.30 and 1.22 (table 4, column 9). These strikingly divergent results were omitted in forming the average. The respiratory quotient of 0.67 on January 10th (table 4, column 7) was also not included in the average. Not only are the differences in the gaseous contents which are $\frac{4.23 \text{ vol. per cent } CO_2}{6.25 \text{ vol. per cent } O_2}$ smaller than the average ratio for the whole series, $\frac{13.75 \text{ vol. per cent } CO_2}{13.91 \text{ vol. per cent } O_2}$, thus making the result less reliable, but the ratio is so far from the average of the series that it is considered reasonable not to include it. Finally the unphysiologic quotients obtained from the expired air which are bracketted in columns 8 and 9 of tables 1, 2, and 4 may have been produced by a fall in the carbon dioxide capacity which may occur as a result of decerebration just as in the case of other operative procedures.

A fall in the carbon dioxide capacity did not cause the respiratory quotient obtained from the blood to differ from that of the expired air of the resting animal. The total volume of carbon dioxide in the blood, that is, the sum of the amounts in solution and in chemical combination, may not be greatly changed though more is in solution and less in chemical combination. Since the total volume of carbon dioxide in the venous blood may be unchanged, the difference in volumes of carbon dioxide between the arterial and venous blood may still represent the increase due to oxidations only, in spite of a change in carbon dioxide capacity. Such a condition obtained in most of the obervations on isolated muscle where the respiratory quotient of the blood of the exercising muscle and the air of the resting animal agreed even though, as in some of the experiments, the respiratory quotient of the air during exercise is higher.

In the case of exercise the amount of extra carbon dioxide liberated in the alveoli may have been influenced by the lactic acid produced by the work. No determinations of lactic acid have been made in the present study, but from a consideration of the literature, and experiments now in progress (Himwich, Koskoff, and Nahum, 1928) we may say the following: As a result of the decerebration there is an outpouring of lactic acid to the

blood stream. It is this lactic acid which causes a fall in the carbon dioxide capacity of the blood from the exercising muscle. It explains the fall in carbon dioxide capacity of the blood on passage through the resting muscles previously observed (Himwich and Castle, 1927) and also the lower capacity usually obtained in succeeding samples of arterial blood both at rest and, as in the present report, during exercise. The greater differences in the carbon dioxide capacities of arterial and venous blood in the isolated muscles may be imputed to the greater severity of an operation involving the removal of the leg, as well as the difference of the preparation itself. In the case of the exercising muscles the conditions for oxidation are improved, due to the changes which are concomitants of exercise resulting in the presence of a greater amount of blood and, therefore, an increased volume of oxygen. Such changes may be accompanied by no increased release of lactic acid into the blood stream, and this is an indication that the "steady state" may be obtained.

It is quite probable that in most of our experiments on the entire leg the "steady state" existed. Aside from the fact that in similar experiments (Himwich, Koskoff and Nahum, 1928) there was no increase in the lactic acid content of the arterial or venous blood, the work was of such mild nature that the venous blood was not greatly reduced in most instances, indicating that the oxygenation of the tissues was sufficient. Furthermore, even though the stronger initial muscular contraction may have hindered the venous return at the beginning of tetanization, yet the fact that the contraction of the muscle rapidly decreased as the stimulation continued shows that this hindrance to return blood flow was quickly diminished. This is borne out by measurements of blood flow made by Castle (personal communication). In an experiment where the muscle was tetanized for 5 minutes, the contraction had become small after the first minute and, at the same time, the blood flow definitely increased. Finally, in a subsequent experiment where the method of stimulation was changed so that there was only one shock per second, allowing ample time for recovery, the respiratory quotients were similar.

The respiratory quotients here presented are more consistent than any previously reported for muscle. Many factors enter into this improvement: In the first place, these experiments retain the good features of those on resting muscle (Himwich and Castle, 1927) inasmuch as muscle *in situ* in the whole animal was used and not excised muscle. This technique excludes the perfusion of the muscles with any foreign substances and prevents any change of concentration of the blood constituents which might occur with perfusion. Secondly, of great importance is the increased gaseous exchange. As the result of exercise in the first series of the present experiments there were 14.0 volumes per cent of carbon dioxide produced and 17.2 volumes per cent of oxygen removed. These values are approxi-

mately three times as great as those obtained during rest (Himwich and Castle, 1927) where the mean deviation of a single respiratory quotient was ±0.16 and the mean error was ±0.13 The present data with analyses good to 0.2 volume per cent, a single respiratory quotient has a deviation of ±0.05 from the mean. The average difference between the 18 double determinations of respiratory quotients appearing in the 7th column of tables 1, 2, 3 and 4 is 0.07. Hence the mean error of a single determination is ±0.035. In the third place, the carbon dioxide capacities in arterial and venous blood were very close in the experiments on the whole lower extremity, thus avoiding the complication of extra carbon dioxide. Furthermore, in the case of the isolated muscle where the venous carbon dioxide capacity was lower, in the majority of the observations the respiratory quotient in the blood agreed with that of the resting animal even though occasionally that in the expired air of the working animal during exercise appears too high. And lastly, the differences in the concentrations of the arterial and venous bloods are small, making unnecessary any correction for changes in concentration.

It should be noted that the respiratory quotients of the fasted and fed dogs as given in table 6 are somewhat higher than might have been expected from the nutritive conditions of the animals. This phenomenon may be the result of the operative procedures occurring previous to the metabolic studies, for decerebration produces hyperglycemia.

Carbon dioxide capacity of the blood. In 37 of 45 observations on the carbon dioxide capacity of arterial and venous blood, the latter showed an average decrease of 1.74 volumes per cent while in 8 observations there was an average increase of 0.71 volume per cent in the venous blood. In 13 of 15 observations, where succeeding samples of arterial blood were drawn, there was an average decrease of 3.27 volumes per cent in the second sample. In the remaining two experiments the average increase was 2.53 volumes per cent. Thus, succeeding samples of arterial blood, taken at an interval of 1 hour, revealed smaller diminutions in carbon dioxide capacity than might have been expected from the decreases occurring on a single circulation through muscle as demonstrated on samples of arterial and venous blood drawn simultaneously.

A liberation of lactic acid from muscle probably caused the decrease in carbon dioxide capacity of the venous blood. However, the carbon dioxide capacity of the arterial blood is the resultant of those of the venous blood returning from all parts of the body. Since organs other than muscle, for example the liver, may remove lactic acid (Himwich, Koskoff and Nahum, 1928) they probably effect an increase of the carbon dioxide capacity of the blood passing through them. These two opposing processes result in the relatively small decrease in carbon dioxide capacity observed in succeeding arterial samples.

Oxygen consumption. From the observations (Himwich and Castle, 1927) on a muscle preparation identical with the one used here, a comparison of the oxygen consumption during rest and exercise is available. Under resting conditions the average consumption of oxygen by an isolated muscle is 0.356 cc. per gram of muscle per hour. For exercise, the average 2.22 cc. is roughly six times as large. The minimum is 1.08 cc. and the maximum 5.04 cc.

Chauveau and Kaufman (1887) whose observations on the levator muscle of the upper lip of a horse varied between 0.168 cc. and 0.474 cc. oxygen per gram per hour while the muscle was at rest, found during exercise an oxygen consumption of 0.6 cc. to 3.0 cc. per gram an hour, values which are somewhat smaller than the present ones. Other investigators have studied anesthetized animals. Barcroft and Kato (1915–16), working on dogs under urethane narcosis, obtained an oxygen intake of 2.64 cc. and 2.94 cc. during intermittent stimulation. The present observations may be compared with the previous ones of Verzar (1912) on urethanized cats since he also used a tetanizing current. Twice he observed a fall in oxygen consumption during the tetanus and twice a rise, though following the tetanization oxygen consumption was always increased. We found an increase in every case except three not included in table 5 because consistent values for blood flows could not be obtained as successive observations yielded decreasing blood flows. The differences in the results may almost certainly be explained by the rate of the blood flow. Whenever Verzar (1912) observed a slower blood flow during the tetanic contraction of the muscle, the oxygen consumption decreased and vice versa. Verzar tetanized the muscles for short periods, 6 seconds in duration from one of his figures. In our experiments there was an increase in the blood flow during tetanus, probably because the observation was taken a longer time after the commencement of the stimulation as described above (p. 674). On resting muscles of dogs, an average venous flow of 0.0015 cc. per gram second was noted (Himwich and Castle, 1927). The present observations disclosed a flow varying between 0.0013 cc. and 0.01114 cc. with a mean of 0.0048 cc.

The data in table 5 permit a comparison between the oxygen consumption of the whole body during rest and the increase in oxygen consumption of the whole body during the exercise of its muscles. Assuming that the muscles weigh 8.88 kilograms, or two-fifths of the average body weight of 22.2 kilos, the average oxygen intake of all the muscles of the body would be 19,613 cc. per hour if they were consuming oxygen at the same rate as the isolated muscle. On the other hand, the average oxygen intake per hour of the whole body at rest is 10,078 cc. Thus the average value for the exercising muscles only would be roughly twice as large as the oxygen intake of the whole body during rest. The maximum oxygen intake dur-

ing exercise occurred in the experiment of March 5th when the oxygen consumed was 5.04 cc. per gram of muscle per hour. If we employ the same calculation as above, we find that the oxygen consumption resulting from exercise was 5 times the resting value. In view of the fact that in the present experiments the muscles were not forced to perform their maximum amount of work, it may be noted that Henderson and Haggard (1925) have found increases of 15 to 20 times the basal oxygen consumption during severe exercise of human beings and, likewise, Furusawa, Hill, Long and Lupton (1924) have found maximum increases of 15 times the resting value, which might mean an intake of 17 cc. per gram of muscle per hour. However, the oxygen consumption of muscles may not give the entire volume of oxygen necessitated by the exercise since it is probable that recovery, with its additional oxygen consumption, occurs not only in muscle but also in other organs (Himwich, Koskoff and Nahum, 1928). In addition, there is extra work by other parts of the body, such as the heart, which is not included in the amount of oxygen consumed by the muscles of the legs.

Oxygen capacity of the blood. Observations on the oxygen capacities of the arterial and venous blood reveal the fact that in 26 of 45 observations there was an average increase of 0.37 volume per cent in the venous blood. In 19 instances there was an average decrease of 0.18 volume per cent. While any changes in the oxygen capacity of the blood, as it passed through the muscles, were usually within the error of the method, yet in the majority of instances the oxygen capacity of the venous blood was raised, pointing to a slight increase in concentration. These results by themselves are not significant. However, they are in agreement with those of Barcroft and Kato (1915–16) who found a slight concentration in exercise. As these authors suggest, the concentration may be caused by the formation of lymph as well as by the absorption of fluid by the working muscles. An increase of pH of the venous blood as it becomes oxygenated would also cause some dilution of the arterial blood.

Apparently, after blood was drawn, in most cases more fluid must have entered the blood to dilute it since in 9 of 15 experiments where two succeeding samples of arterial blood were drawn, the second observation revealed an average decrease in oxygen capacity of 1.03 volumes per cent. If additional red blood corpuscles were entering the circulating blood in these 9 experiments, their rate of entrance was slower than that of their suspending fluid. However, in the remaining 6 experiments an average increase of 0.79 volume per cent was noted.

SUMMARY AND CONCLUSIONS

The respiratory quotient of exercising mammalian muscle as determined by quantitation of the gases in the blood in the vessels of the active parts

was compared with the respiratory quotients of the whole body during rest and exercise as determined by analysis of the expired air.

Forty-nine experiments were performed on thirty decerebrate dogs. The experiments fall into two major groups, one on dogs fasted from 5 to 15 days and another on dogs fed a diet containing considerable carbohydrate. In certain animals in each of these two groups the venous blood was drawn directly from the femoral vein carrying the blood returning from the whole lower extremity, and in others the venous blood was drawn after the circulation of the gastrocnemius and flexor digitorum sublimis muscles had been isolated.

The averages of the various respiratory quotients were as follows: directly from the blood of the non-isolated muscles of the lower extremity of fasted dogs -0.81 ± 0.05 during exercise, and for the expired air 0.81 ± 0.03 on the resting animals, and 0.83 ± 0.05 on the exercising animals; for the isolated muscles of fasted dogs -0.78 ± 0.06 during exercise, and 0.80 ± 0.03 and 0.96 ± 0.07 for the resting and working intact animals respectively; for the muscles of the lower extremity of fed dogs -0.91 ± 0.04 during exercise and 0.90 ± 0.02 on resting dogs and 0.95 ± 0.02 on working dogs, and finally for the isolated muscles of fed dogs -0.98 ± 0.07 during exercise and 0.94 ± 0.02 and 1.06 ± 0.10 for the resting and working animals respectively. In view of the close agreement between the respiratory quotients at rest and those in exercise, it is concluded that exercising muscle from the second to the fifteenth minute of exercise utilizes foodstuffs in approximately the same proportions as the whole body during rest.

A study of the carbon dioxide and oxygen capacity values of the arterial and venous blood samples from which the respiratory quotients of exercise were determined, revealed the following: 1. A slight concentration of the venous blood, usually within the error of the method, took place in its passage through the exercising muscles. However, the succeeding sample of arterial blood usually showed a slight dilution. 2. In the majority of observations on the muscles of the whole lower extremity but slight changes in the carbon dioxide capacities were apparent, while the carbon dioxide capacities of the venous blood were usually lower than the arterial in the experiments on the isolated muscles. The decrease in carbon dioxide capacity of the venous blood caused relatively only a slight diminution in the carbon dioxide capacity of a succeeding sample of the arterial blood.

Lastly, the oxygen consumption of the isolated muscle was studied during exercise. The average consumption was 2.22 cc. of oxygen per gram of muscle per hour, and the maximum value for the moderate exercise employed in the present experiments was 5.04 cc. per gram of muscle per hour.

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BIBLIOGRAPHY

ANDERSON, R. J. AND G. LUSK. 1917. Journ. Biol. Chem., xxxii, 421.
BARCROFT, J. AND T. KATO. 1915–16. Phil. Trans. Roy. Soc. London, ccviiB, 149.
BENEDICT, F. G. AND E. P. CATHCART. 1918. Carnegie Institution, Washington, pub. no. 187.
CHAUVEAU, A. 1896. Compt. Rend., cxxii, 1163.
CHAUVEAU, A. AND KAUFMANN. 1887. Compt. Rend., civ, 1763.
FENN, W. O. 1928. This Journal, lxxxv, 207.
FURUSAWA, K., A. V. HILL, C. N. H. LONG AND H. LUPTON. 1924. Proc. Roy. Soc. London, xcviiB, 167.
HENDERSON, Y. AND H. W. HAGGARD. 1925. This Jornal, lxxii, 264.
HIMWICH, H. E. AND W. B. CASTLE. 1927. This Journal, lxxxiii, 92.
HIMWICH, H. E., Y. D. KOSKOFF AND L. H. NAHUM. 1928. Proc. Soc. Exp. Biol. and Med., xxv, 347.
KROGH, A. AND J. LINDHARD. 1920. Biochem. Journ., xiv, 290.
MARSH, E. 1928. Journ. Nut., i, 57.
MEYERHOF, O. 1920. Pfluger's Arch., clxxxii, 284.
RAPPORT, D. AND E. P. RALLI. 1928. This Journal, lxxxiii, 450.
RICHARDSON, H. B. AND S. Z. LEVINE. 1925. Journ. Biol. Chem., lxvi, 161.
SCHMIDT, C. F. 1923. Journ. Exper. Med., xxxvii, 43.
VAN SLYKE, D. D. AND J. M. NEILL. 1924. Journ. Biol. Chem., lxi, 523.
VERZAR, F. 1912. Journ. Physiol., xliv, 243.
WILSON, J. R., S. Z. LEVINE, H. B. RIVKIN AND F. BERLINER. 1927. Amer. Journ. Dis. Child., xxxiii, 618.
ZUNTZ, N. 1911. Oppenheimer's Handbuch d. Biochemie. Jena, iv, 849.

THE RESPIRATORY QUOTIENT OF TESTICLE

HAROLD E. HIMWICH AND LOUIS H. NAHUM

From the Laboratory of Physiology, Yale University, New Haven

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The testicle appears more suitable than other glands for a study of the respiratory quotient by the determination of the gaseous exchange in the blood entering and leaving an organ. Some organs, like the submaxillary gland (Barcroft, 1900) or the intestine (Brodie and Vogt, 1910), may be secreting or absorbing fluids and thus produce changes in the concentration of the constituents of the blood passing through them, making the significance of a comparison of the carbon dioxide and oxygen contents of the arterial and venous blood questionable.

Most glands have vascular supplies which are rich in proportion to their oxygen requirements. In the case of the kidney, Barcroft and Brodie (1904–05) often found such small differences of carbon dioxide and oxygen in samples of arterial and venous blood that a study of the respiratory quotient by this method seemed impossible.

As noted by Schmidt (1928), the variations in carbon dioxide production usually have been greater than those of oxygen consumption. This greater variability in the formation of carbon dioxide may be due to the fact that the perfusion of tissues with the blood of another animal, or blood rendered uncoagulable by heparin (Hayman and Schmidt, 1928) (Rapport and Katz, 1927), or by other means such as hirudin (Barcroft and Dixon, 1906–07), may produce changes such as those in the acid base equilibrium and thus render comparisons of the gaseous exchange unreliable. We were therefore limited to the study of a gland which was not actively engaged in secretion or in absorption, whose blood supply came unaltered from the animal itself and, moreover, was not so large as to make a comparison of the carbon dioxide and oxygen exchanges unreliable. A study of the testicle fulfilled these three requirements. Hence, this organ was used for the present observations. The testicle, moreover, lends itself to such examination because its blood supply is isolated and accessible. In the course of the work, data of an approximate character were obtained on the blood flow and oxygen consumption of the gland.

METHOD. The dogs used for this study were fasted for periods varying between 14 hours and 8 days. After decerebration, which was employed in order to eliminate the necessity for anesthesia, expired air was collected through a tracheotomy tube in a spirometer so that samples of expired air

could be analyzed for carbon dioxide and oxygen in order to determine the oxygen consumption and respiratory quotient of the entire animal. Then a left lumbar incision was made, disclosing the left kidney and left spermatic vein which could be followed to the point where it entered the left renal vein. Occasionally smaller veins from the perirenal fat entered the spermatic vein in which case they were ligated.

Blood was collected and blood flow was estimated in the following manner: With a needle of diameter almost the size of that of the spermatic vein, a sample was drawn under oil in a 10 cc. Luer syringe. On the insertion of the needle and drawing the blood the vein collapsed at the orifice of the needle. No more was drawn until the lumen opened again due to

TABLE 1

Carbon dioxide and oxygen contents of arterial and venous blood of testicle, and respiratory quotients of testicle

DATE	CO_2		O_2*		BLOOD R.Q. OF TESTICLE
	Arterial	Venous	Arterial	Venous	
1927					
January 21	36.09	41.53	21.27	15.43	0.93
March 18	26.71	30.80	23.01	17.43	0.73
June 30	39.58	44.72	23.15	17.78	0.96
July 7	23.61	29.54	29.00	22.26	0.88
August 23	36.08	46.45	22.50	10.03	0.83
	38.15	43.26	22.48	14.39	0.63
August 25	24.65	33.89	23.87	12.96	0.85
November 18	29.47	34.64	26.74	20.46	0.82
	29.64	31.41	27.27	22.11	(0.34)
December 12	12.17	28.92	26.54	10.04	1.02
December 14	31.27	36.05	26.23	19.38	0.70
1928					
October 11	29.90	37.13	24.76	16.39	0.87

* Includes dissolved oxygen in addition to oxygen in combination with hemoglobin.

the presence of a new supply of venous blood. Thus, the blood was collected without stasis and obviously no more rapidly than it passed through the gland and appeared in the vein. A quantity of blood, usually less than 10 cc., was collected and estimated to $\frac{1}{2}$ cc. The complete process usually took from 2 to 6 minutes and was timed with the aid of a stopwatch. A sample of arterial blood was drawn simultaneously from the femoral artery. Both samples were stored in glass containers over mercury. The glass containers had had previously dusted into them enough potassium oxalate and sodium fluoride, corrected to pH 7.3, to make concentrations of 0.2 and 0.1 per cent respectively with 20 cc. of blood. In four observations the blood was exposed to a mixture of $5\frac{1}{2}$ per cent carbon dioxide and oxygen saturated in water vapor at 38°, in order to determine

the carbon dioxide and oxygen capacities. All blood samples were analyzed for carbon dioxide and oxygen by the method of Van Slyke and Neill (1924). Checks were obtained to 0.2 volume per cent.

EXPERIMENTAL. In table 1 may be found the results of the blood gas analyses and the 12 respiratory quotients of the gland; 11 respiratory

TABLE 2

Blood flow of testicle, and oxygen consumption of testicle and of entire animal

DATE	WEIGHT OF DOG	WEIGHT OF TESTICLE	BLOOD FLOW PER GRAM AND MINUTE	O_2 CONSUMPTION PER GRAM AND MINUTE OF TESTICLE	O_2 CONSUMPTION PER GRAM AND MINUTE OF ENTIRE ANIMAL
1927	*kgm.*	*grams*			
January 21	20				
March 18	15	12.9	0.163	0.0091	0.0062
June 30	27	21.6	0.116	0.0062	0.0038
July 7	26.7				0.0062
August 23	20.6	23.5	0.064	0.0080	0.0051
			0.064	0.0052	
August 25	19	15.5	0.116	0.0127	0.0054
November 18	23.3	21.9	0.137	0.0086	0.0083
			0.132	0.0068	
December 12	19.75	17	0.056	0.0092	0.0060
December 14	29.5				0.0043
1928					
October 11	37	34	0.118	0.0098	0.0039
Average.....	23.8	20.9	0.107	0.0084	0.0055

TABLE 3

Carbon dioxide and oxygen capacity of arterial and venous testicular blood

DATE	CO_2		O_2	
	Arterial	Venous	Arterial	Venous
1928				
October 11	31.09	26.73	26.37	27.63
	31.51	31.34	25.67	27.21
October 25	38.98	38.62	25.76	26.84
	38.81	40.45	26.31	26.96

quotients are within the physiologic range. The second respiratory quotient of November 18th is probably faulty because of air bubbles in venous sample. On August 23rd succeeding respiratory quotients on the same preparation are not in agreement.

Table 2 contains the data on the blood flow and oxygen consumption of

the testicle as well as the oxygen consumption of the entire animal. Variations in blood flow extend from 0.056 to 0.163 cc. per gram of tissue per minute, with an average of 0.107 cc. per gram and minute. The oxygen consumption of the testicle varies between 0.0052 cc. and 0.0127 cc., with a mean of 0.0084 cc. per gram of tissue per minute. The oxygen consumption of the whole animal is 0.0055 cc. per gram of tissue per minute. The average weight of the dogs is 23.8 kilos and that of the testicles 20.9 grams.

In table 3 are presented the results of four observations on the carbon dioxide and oxygen capacities of the arterial and venous blood of the testicle. The values for carbon dioxide capacity show no consistent changes. In the first observation, the carbon dioxide capacity is definitely lower in the venous blood than in the arterial. In the second and third determinations, the capacities remain practically the same while in the remaining instance the carbon dioxide capacity in the venous blood is higher than in the arterial.

The oxygen capacity of the venous blood is greater than that of the arterial in all four observations. Apparently the blood concentrates on passage through the testicle.

Discussion. The chief fact which emerges from the above results is that the group of respiratory quotients of the testicle falls within the physiologic range, indicating the combustion of the usual combinations of foodstuffs. The variations in the respiratory quotients might be expected since the several experimental animals were not under the same nutritive conditions. Moreover, decerebration may produce hyperglycemia of varying intensities.

In evaluating the individual respiratory quotients at least three factors should be taken into consideration—the carbon dioxide capacity of the blood, its concentration, and the analytical error.

It is not known how changes in the carbon dioxide capacity of the blood may affect the respiratory quotient. However, in 3 of the 4 observations the differences are probably too small to be of importance.

On the other hand, a relatively increased concentration of the venous blood may lower the respiratory quotient. Such a concentration occurred in each of four observations and may be a constant phenomenon. In the second observation of October 11th, data were obtained not only for the concentration of the blood but also for the respiratory quotient. Since the maximum change, 1.54 volumes per cent, in the concentration of the blood occurred in this observation it becomes of special interest to determine to what extent such a change may alter the respiratory quotient. Applying corrections previously described (Himwich and Castle, 1927) the respiratory quotient of 0.87 (table 1, Oct. 11, 1928) is lowered to 0.82.

The analytical error may be determined quite accurately. If the second observation of November 18th is omitted, the average difference in carbon

dioxide between arterial and venous blood is 7.20 volumes per cent and that for oxygen is 8.45 volumes per cent. With an error of analysis of 0.2 volume per cent that of the respiratory quotient is ± 0.045 from this source alone. Taking the combined errors into consideration it is probable that the respiratory quotients indicate approximately the proportion of foodstuffs burned.

The respiratory quotients obtained by analysis of the expired air are not published because the expired air was collected before the first effects of the operative procedures had worn off. Hence, the present data yield no information regarding a relationship between the respiratory quotient of the testicle and that of the whole animal though it might be expected that there would be an agreement between them.

Blood flow and oxygen consumption of the testicle. Both blood flow and oxygen consumption of the testicle are larger than those of resting muscle (Himwich and Castle, 1927) which are 0.084 cc. per gram and minute and 0.0058 cc. per gram and minute respectively. Probably the actual oxygen consumption of the testicle is somewhat larger than the apparent value. If the increased concentration of the blood is taken into account in the second observation of October 11th, the observed oxygen consumption of 0.0098 cc. per gram and minute is increased to 0.0110 cc. per gram and minute. In any event, the oxygen consumption of the testicle per gram and minute is greater than that of the whole animal per gram and minute, 0.0055 cc. The latter figure is somewhat lower than that obtained in previous work (Himwich and Castle, 1927) (Himwich and Rose, 1929).

Oxygen capacity of testicular blood. The concentration of the venous blood is probably due, in large part, to a loss of fluid to the lymphatics.

SUMMARY. The gaseous exchange of the testicle in the body has been studied by analyses of samples of its arterial and venous blood for carbon dioxide and oxygen. Fifteen pairs of samples were analyzed from 11 dogs and, from the data thus obtained, 12 respiratory quotients of the testicle were calculated and approximate values for the blood flow and oxygen consumption of that organ were determined. In addition, four observations were made of the carbon dioxide and oxygen capacities of arterial and venous testicular blood. Lastly, the respiratory metabolism of the experimental animals was studied.

CONCLUSION

The respiratory quotients of the testicle fall within the physiologic range, indicating that this organ oxidizes the usual combinations of foodstuffs.

BIBLIOGRAPHY

BARCROFT, J. 1900. Journ. Physiol., xxv, 479.
BARCROFT, J. AND T. G. BRODIE. 1904–05. Journ. Physiol., xxxii, 18.
BARCROFT, J. AND W. E. DIXON. 1906–07. Journ. Physiol., xxxv, 182.
BRODIE, T. G. AND H. VOGT. 1910. Journ. Physiol., xl, 135.
HAYMAN, J. A., JR. AND C. F. SCHMIDT. 1927–28. This Journal, lxxxiii, 502.
HIMWICH, H. E. AND W. B. CASTLE. 1927–28. This Journal, lxxxiii, 92.
HIMWICH, H. E. AND M. I. ROSE. 1929. This Journal, lxxxviii, 663.
RAPPORT, D. AND L. N. KATZ. 1927. This Journal, lxxx, 185.
SCHMIDT, C. F. 1928. This Journal, lxxxiv, 223.
VAN SLYKE, D. D. AND J. M. NEILL. 1924. Journ. Biol. Chem., lxi, 523.

THE MEASUREMENT OF MUSCULAR TENSION AND ITS BEARING ON PLURI-AXONAL INNERVATION

H. C. STEVENS AND ENOCH KARRER

From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University

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The problem of the ultimate mode of distribution of the constituent fibers of a motor nerve to the constituent fibers of a skeletal muscle has interested anatomists and physiologists for many years. The attack on the problem by modern experimental methods may be said to have begun with the publication by Johannes Gad (1882) of his classical paper on the relations between nerve, muscle and center. Since then the subject has been studied both by anatomical and physiological methods. The literature on the question has been recently reviewed by Fulton (1926) and by Weed (1927). The general conclusion, reached by anatomists, appears to be that in some instances branches of the same axone may innervate singly several muscle fibers and in other instances, doubly innervate certain muscle fibers. The problem has usually been stated with reference to the unisegmental or plurisegmental origin of the nerves. However, the question as to whether muscle fibers are doubly innervated may be discussed without reference to the spinal origin of the nerves. We have chosen to consider the problem from this point of view using the terms *uni-axonal* and *pluri-axonal* innervation to designate respectively the anatomical conditions in which a muscle fiber is innervated by one nerve fiber and by two or more fibers derived from different axones. Possible relationships between axonal and segmental innervations are illustrated in figure 1.

We were induced to take up the study of this problem because of certain questions which arose in connection with the conduction of the nerve impulse through a zone of narcosis. It became evident, when repeating some of Adrian's experiments on the conduction of nerves narcotized by alcohol, that the determination of the end point of the reaction by means of the muscular contraction could not serve as a true indication of the narcosis of the nerve trunk, fiber by fiber, unless a one to one relationship exists between nerve fiber and muscle fiber. For it is obvious, to assume the extreme case, that if all the fibers of a muscle are doubly innervated, 50 per cent of the nerve fibers composing a nerve trunk could be narcotized without affecting the response of the muscle, provided that both of the

innervations were equally potent to excite the muscle fiber. Since, in experiments of this sort the muscle response is employed to indicate what is going on in the nerve, it would seem to be necessary first to understand the nature of the innervation of the muscle.

The special anatomical condition required by the physiological method is a muscle which derives its nerve supply from two separate sources such as the gastrocnemius of the frog, which is innervated chiefly from the 8th and 9th lumbar roots or the crico-thyroid muscle which is innervated by the middle and superior laryngeal nerves. It may be pointed out that it is possible to split a motor nerve close to its point of entrance into the muscle and so create the anatomical condition required by this method.

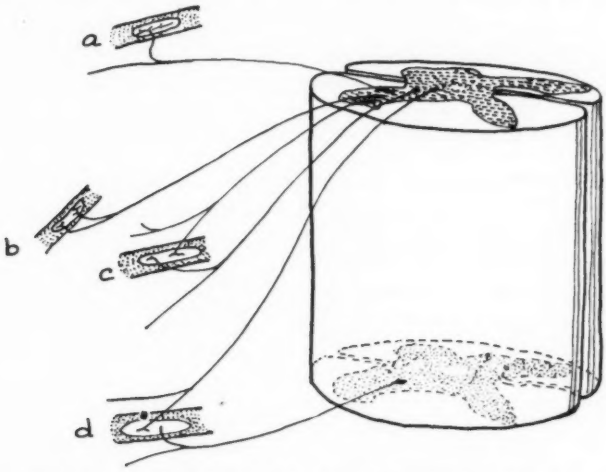

Fig. 1. *a*, uni-axonal, unisegmental; *b*, double innervation from one axone; *c*, pluri-axonal, unisegmental; *d*, pluri-axonal, plurisegmental.

Since the two sets of axones enter the muscle grossly at the same point, it may be suggested that the possibility of overlap might be greater than if the nerves enter at different points as do the branches of the sciatic which supply the gastrocnemius. This procedure makes possible the use of any motor nerve and any muscle.

With such an anatomical mechanism, the response of the muscle measured as mechanical tension, action current or heat is determined when the muscle is excited by stimulation of one nerve alone, then of the other alone and finally of both together. Three measurements are thus made and their bearing upon the pluri-axonal question depends upon the relation of these three quantities to one another. The following symbols represent the quantities and their relations. Let *a* stand for one, and *b* the other

nerve source; R for the response of the muscle (mechanical tension, action current or heat); R*a*, R*b* and R*ab* the response of the muscle when excited respectively by stimulation of *a* and *b* separately and simultaneously. The sum of R*a* and R*b* then is R*a* + R*b*. There are three possible quantitiative relations between R*a* + R*b* and R*ab* viz., R*a* + R*b* $\begin{matrix} > \\ = \\ < \end{matrix}$ R*ab*.

The results reported by investigators of these three alternatives have varied with the experimenter and the type of measurement made. In general the measurement of action current and heat has yielded approximate equality of R*a* + R*b* and R*ab*. Measurement of tension, on the other hand, has in general resulted R*a* + R*b* > R*ab*. There have been some notable exceptions, as in the early experiments of Gad (1882) which were made with a Fick isometric lever, and in those of Quednau (1926). Usually, however, R*ab* has been reported from 11 per cent to 50 per cent less than

TABLE 1

Tension in kilograms

	ORDER OF STIMULATION	L	R	L + R	B	$\frac{B}{L \text{ AND } R}$
						per cent
Experiment 1	L-R-B	2.070	3.560	5.360	5.090	90.4
Experiment 2	L-R-B	1.141	3.080	4.221	4.240	100.4
Experiment 3	B-L-R	0.933	2.590	3.523	3.715	105.4
Average						98.7

R*a* + R*b*. Such results have been interpreted to mean that a certain percentage of the muscle fibers are doubly innervated by fibers from both *a* and *b*. This inference implies the assumption that a muscle fiber doubly innervated behaves like one singly innervated, irrespective of whether it be stimulated in any one of several ways, such as through either one of the nerve fibers alone or through both simultaneously, or through both alternately.

A brief résumé of the literature will suffice to show that the results of measurement of muscular tension have, in general, not been additive while measurement of action potential and heat yield approximate summation.

Gad (1882) using a Fick isometric lever and the gastrocnemius of the frog found that tension was 100 per cent additive. Exner (1885) employing the crico-thyroid muscle which he found to be doubly innervated by the superior and middle laryngeal nerves, attempted to determine the amount of overlapping by section of one nerve and observing the subsequent degeneration in the muscle. His results were incon-

clusive. Lederer and Lemberger (1907) using the same preparation and the Fick lever obtained 100 per cent additivity of the muscular tension. Their results with the flexor digitorum of the rabbit failed to give summation. Agduhr (1916, 1919) renewed interest in the problem by histological studies using the method of nerve degeneration and also by the measurement of tension. The latter experiments gave from 55 per cent to 60 per cent summation. Cattell and Stiles (1924, a, b) measured muscular tension in the gastrocnemius of the frog and obtained an additivity of about 70 per cent. Samojloff (1924, 1925) measuring simultaneously the action potential and the tension of the frog's gastrocnemius found 102.4 per cent for the former and 80 per cent for the latter. Katz (1925) obtained 77 per cent for tension and 101 per cent for heat in experiments made with the gastrocnemius of the frog. De Boer (1926) reported 100 per cent additivity in measurements of action potential of the gastrocnemius of the frog. Boyd (1926) attempted to correlate a low additivity (23 per cent with the triceps surae of the frog) with the degree of obliquity of the muscle fibers in pinnate muscles. Fulton (1926) making use of the torsion wire myograph and the gastrocnemius of the frog obtained a summation of 89 per cent. Quednau (1926) obtained 100 per cent additivity with the flexor digitorum of the rabbit. Cattell (1928) employing the sartorius of the frog measured tension, under isometric conditions, by means of the torsion wire myograph with the result that the combined tension varied from 53 to 88 per cent of the sum of the separate tensions.

In our experiments the lateral head of the gastrocnemius muscle of decerebrate cats was employed. Decerebration was performed by transecting the brain stem by means of a stiff wire (under ether anesthesia and after ligation of the carotid arteries), through a small trephine opening, except in some experiments where a thalamus preparation was desired, a special knife (Karrer and Stevens, 1928a) designed for this purpose was used. It is possible, in the cat, to separate the medial head of the gastrocnemius from the lateral and by ligating and amputating the former, to leave the lateral head intact with two branches of the tibial nerve entering it at different points. A thin band of muscle tissue along the median line of the dorsal surface of the muscle is severed. When there is bleeding at this point, it is controlled by ligation. The two branches *a* and *b* of the tibial nerve enter the muscle at different points, *a* entering on the medial surface of the muscle about 2 cm. from the proximal end, and *b* in the mid-line of the dorsal aspect about one centimeter from the origin of the muscle. The former innervates mainly the dorsal and medial portion of the muscle, the latter chiefly the lateral and inferior portion. This preparation presents certain advantages for the study of muscle nerve problems in mammals. Circulation in the muscle is little disturbed and the animal survives on the average for about six to eight hours. The sciatic nerve is accessible for several centimeters and the two branches from the tibial nerve which enter the lateral head can easily be freed, for a distance of 2 cm. from the muscle. The large femur by which the leg may be clamped and the large tendo achillis are useful for purposes of fixation and traction. To avoid loss of body heat and drying, electric lamps are placed under the animal and the muscle and nerve kept moistened with

Ringer's solution. With the electrodes placed in the middle of a portion of nerve about 2 cm. in length, current spread was tested by section of the peripheral end of one nerve and maximal stimulation of the central end of the same nerve to observe whether there was response through the intact nerve and its portion of muscle. It is essential to avoid such sources of error as insecure fixation of the femur, the slipping, lengthening, cutting and tearing at the hook which is inserted through the tendon. This hook is wrapped and bound to the tendon and bone by means of twine. The femur is exposed and firmly gripped by the teeth of the clamp which would penetrate any layer or cushion of tissues. The tendon and the opposing spring are attached to the pulley system by means of several strands of fine, flexible steel wire. The lever system magnified 5.47 times. Since, in our experiments, it was possible to record and observe, with reasonable

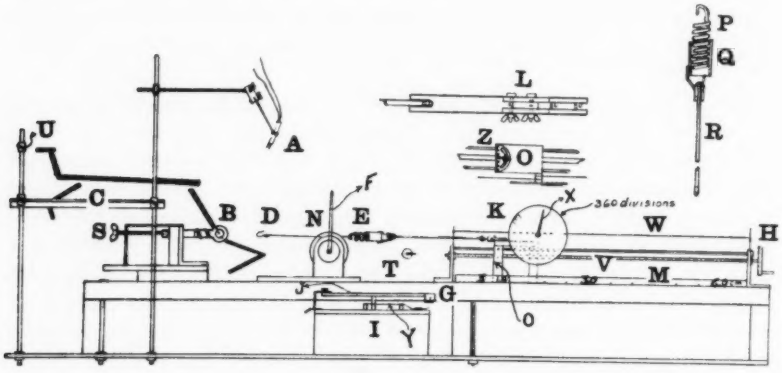

Fig. 2

certainty, approximately 0.1 mm., the muscle was isometric to within about 0.02 mm. at the moment when it exerted its maximal force.

Apparatus. The apparatus, which was developed after much preliminary experimentation necessary to overcome the many technical difficulties associated with measurement of muscular tension, is shown in some detail since with improvement of the apparatus we were enabled to obtain more satisfactory experimental results. The general arrangement of the various parts seen from the side is shown in figure 2, in which a decerebrate cat is represented by the heavy black line as lying belly down on a canvas frame *C* with its head supported by a head holder, *U* (Karrer and Stevens, 1928b). The femur is exposed and clamped in a bone clamp, *B* (insert *L*). Two such clamps are mounted side by side, separated sufficiently to permit the simultaneous use of both legs. Four

electrodes, of which one only, *A*, is shown, are available. The tendon is attached to the lever system, *N*, by means of a hook or tendon clamp (Karrer and Stevens, 1928c). The stylus, *F*, is adjusted to write on a drum (not shown) which is carried on the disc, *J*, of a phonograph motor, *I*. The nerves are excited by an automatic stimulator, *G*, consisting of spring brushes which make contact across two wires, *Y*, at each revolution. The duration of stimulation can be varied by lengthening these wires or changing the speed of the motor. A time marker, *T* (Karrer and Stevens, 1928d) giving a time interval of approximately $\frac{1}{20}$ second, is adjusted to write on the drum under the stylus and in alignment with the signal marker which is not shown. The contracting muscle is opposed by the adjustable spring, *E*, (insert *PQR*) one end of which is attached to the lever system, *N*, and the other to the traveling carriage, *O* (insert *ZO*, top view). Tension of the spring is increased or diminished by turning the screw, *V*, by means of the disc, *H*. Readings are made on the scale, *M*, for coarse, and on the dial, *K*, for fine adjustments. The pointer, *X*, of the dial is rotated by means of the wire, *W*, which encircles a cylinder mounted upon the carriage, *O*, to which the pointer is attached. Each degree on the dial corresponds to 0.12 mm. of travel of the carriage, *O*.

Early in these experiments it was found, as others have found, that the more nearly isometric the conditions were and also in general the stiffer the spring which was used to oppose the muscle, the more perfect was the additivity. This became quite striking while obtaining myograms with different springs and under different conditions. In figure 4 are plotted the elastic constants of the springs used (abscissae) and the per cent of additivity (ordinate) which was obtained. This graph shows that there is a high degree of correlation between stiffness of spring and high additivity of muscular response. At the moment when the maximal tension is recorded, the muscle is isometric to the same extent in both cases. The spring was adapted to the particular animal used in an experiment, by means of the following method. First a long spring (22 cm. long, stretching 1.55 cm. for each kilo) was used and, with an initial load of 50 grams to 80 grams, the maximal shortening of the muscle was determined with the least maximal contraction stimulus (L.M.C.S.). With the same spring, the maximal load of the muscle under isometric conditions was determined. Two limiting conditions were thus measured, viz., maximal shortening with minimal load and maximal load with minimal shortening. The third step was to calculate that length of the adjustable spring (fig. 2, *E* and *PQR*) which would be stretched a distance equal to the maximal shortening under minimal load by the maximal load determined under isometric conditions. For example, if the maximal shortening under the initial load was 1 cm. and the maximal force developed under isometric conditions was 10 kgm., we determined by calculation, from the

elastic constants, the length of the adjustable spring which would be extended 1 cm. under a load of 10 kgm. Springs selected in this manner gave good additivity and most of our observations were made by such springs. In five experiments, the spring was deliberately lengthened after good additivity had been obtained with a shorter spring. This change always resulted in poor additivity (cf. fig. 3 the points indicated by *S*). The results obtained in such experiments are not included in table 2.

To determine the range of experimental error inherent in this type of experiment, with our apparatus and technique, the entire lateral head of the gastrocnemius and the tibial nerve of each leg were used instead of one muscle and the branches of the nerve. With two separate legs, there can be no question of a common field of innervation. The sum of the tensions developed in the two muscles excited separately should approximately

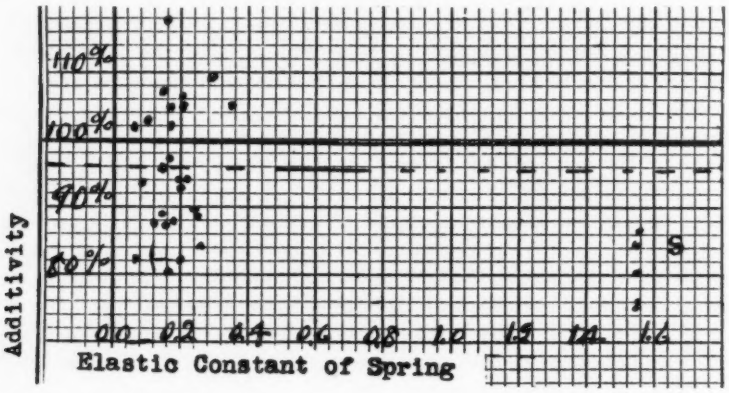

Fig. 3

equal the tension of the two muscles excited together. R*a* + R*b* should equal R*ab*. Typical results are given in table 1 where three measurements are recorded with the lateral heads of the right and left gastrocnemii of a cat. The letters L, R and B refer to the left, right and both lateral heads of the gastrocnemii, respectively. It will be seen that the range of experimental error is about 5.5 per cent.

In our experiments we first determined the least maximal contraction stimulus (L.M.C.S.). The stimulus which had a duration of 0.12 second was produced by the secondary coil of a Harvard inductorium, the primary of which was activated by a two volt storage battery. The L.M.C.S. was first obtained, the secondary coil usually requiring to be 11 cm. from the primary and tilted about 30° from the vertical. We next determined the optimal, initial, passive extension. This was accomplished by turning

TABLE 2

Tension in kilograms

DATE	ORDER OF STIMULATION	Ra	Rb	Ra + Rb	Rab	ADDITIVITY $\frac{Rab}{Ra + Rb}$
						per cent
December 5, 1926	ab-b-a	1.787	2.403	4.190	4.072	97.1
December 8, 1926	ab-b-a	0.470	0.170	0.640	0.700	109.3
December 10, 1926	b-a-ab	1.460	2.440	3.900	4.100	106.0
December 12, 1926	b-a-ab	4.330	6.380	10.710	9.260	86.4
	b-a-ab	4.420	6.020	10.440	9.360	89.6
December 19, 1926	b-a-ab	3.635	4.934	8.569	9.112	106.3
	b-a-ab	3.906	5.663	9.569	9.112	95.2
	ab-b-a	3.953	5.355	9.308	9.570	102.8
January 21, 1927	a-b-ab	2.377	3.118	5.495	5.468	99.4
	b-a-ab	1.944	1.839	3.783	3.524	92.9
January 30, 1927	b-a-ab	5.313	4.281	9.594	8.940	93.1
February 6, 1927	b-a-ab	5.025	7.193	12.218	10.130	82.9
	b-a-ab	1.016	2.280	3.296	2.941	89.2
February 13, 1927	ab-b-a	6.111	8.895	15.006	15.130	100.7
	a-b ab	7.350	10.110	17.460	20.000	114.5
February 20, 1927	ab-b-a	1.050	2.068	3.121	3.250	104.0
February 27, 1927	ab-b-a	2.426	4.769	7.195	6.661	92.5
	ab-b-a	1.553	4.274	5.827	5.553	95.1
March 6, 1927	ab-b-a	4.292	7.091	11.383	11.100	97.5
	ab-b-a	3.896	6.317	10.213	10.410	101.9
	ab-b-a	3.012	4.621	7.633	7.993	104.7
	a-b-ab	2.658	3.243	5.901	4.743	80.3
	ab-a-b	1.347	1.615	2.962	3.518	118.7
March 13, 1927	ab-b-a	2.134	4.173	6.307	6.668	105.7
	a-b-ab	1.908	3.735	5.643	4.629	81.9
	ab-b-a	1.557	3.120	4.677	4.403	94.1
	a-ab-b	1.514	2.442	3.956	3.711	93.8
March 20, 1927	b-ab-a	2.239	8.521	10.760	9.510	88.3
	a-ab-b	2.135	7.772	9.907	8.500	85.8
April 10, 1927	b-ab-a	4.606	8.928	13.534	12.040	88.6
	b-ab-a	3.128	8.931	12.059	10.840	89.8
	ab-b-a	3.863	8.230	12.093	10.070	83.2

TABLE 2—*Concluded*

DATE	ORDER OF STIMULATION	Ra	Rb	Ra + Rb	Rab	ADDITIVITY $\frac{Rab}{Ra + Rb}$
						per cent
April 24, 1927	ab-b-a	4.450	7.231	11.681	11.700	100.1
	ab-b-a	2.814	4.874	7.688	8.281	107.5
	a-b-ab	3.244	5.437	8.681	8.577	98.8
	a-b-ab	2.888	4.903	7.791	7.688	98.6
	ab-b-a	2.755	4.770	7.525	7.644	101.3
May 1, 1927	ab-b-a	6.629	8.388	15.017	12.530	83.4
	a-b-ab	6.747	8.388	15.135	14.020	92.6
	ab-b-a	6.458	7.423	13.881	12.100	87.1
Average						96.0

Fig. 4 (reduced to $\frac{2}{3}$ of original)

the screw, *S*, (fig. 2) of the bone clamp while, by adjusting the screw, *V*, by means of the handle on the disc, *H*, (fig. 2) of the dynamometer, the extension indicator, *N*, was kept at its stop and the stylus at zero. It is thus evident that the lever was after loaded. There is therefore a slight, constant tension on the muscle. This tension, in some cases, was measured by means of weights hung over the pulleys and found to vary from 50 to 80 grams. An interval of 20 seconds was allowed between each successive contraction in determining the maximal tension of the fractions or of the total muscle, with a rest of 3 minutes between experiments. The lengths of the muscles varied in different animals from about 9 to 11 cm. The order of stimulation is indicated in the second column of table 2.

The graphic record of a typical experiment is shown in figure 4. The measurements in grams of this experiment are given in table 2 under the date of April 24, 1927. In figure 4, the first line, *1*, indicates the maximum contraction of the muscle, with a long spring, which is the first step in selecting the proper spring. The first group, *2*, of four marks was made in determining the L.M.C.S. The second group, *3*, of lines was made in determining the maximal tension of the muscle when both nerves were simultaneously excited. The first mark in this group indicates the height of contraction with the initial load. Seven other contractions successively decreasing with increasing loads were recorded before the end point and the maximal tension were reached. The last line in this group is the record of a test contraction made again with the slight initial load for comparison with the first, in order to see how well the reactivity of the preparation had maintained itself through the series of contractions necessary for determining the maximal load. These two contractions taken before and after a determination of maximal tension usually did not differ much. The test contraction was sometimes higher than the first. When it was materially lower than the first the preparation was usually a poor one. These contractions were obtained under conditions of equal length of muscle. The actual tension in the opposing spring in equilibrium with that in the muscles may vary slightly. Usually, however, the change in the zero condition before and after, which is a measure of the change in the tension was not more than 2° or about 0.24 mm. Similarly, group *4* refers to the maximum tension of the muscle when one nerve branch, *a*, is stimulated and group *5* when the other, *b*, is stimulated. Determinations of maximum tension during these contractions yielded additivity of 100.1 per cent. Groups *6*, *7* and *8* yielded 107.5 per cent with a slightly shorter spring; *9*, *10* and *11*, yielded 98.8 per cent. After some adjustments of the electrodes were made, groups *12*, *13* and *14* were recorded with a spring eighteen times as long as the first one (to observe the influence of using a much longer spring), yielding 84.8 per cent. Groups *15*, *16*, *17* and *18*, *19*, *20* were obtained again using the short spring and yielded 98.6 per cent and 101.3 per cent, respectively.

In table 2 are summarized the results of forty experiments performed with the apparatus and technique described. The maximum tension, R*a* in kilograms, measured when the muscle was stimulated through one nerve branch, *a*, is shown in the third column, the tension R*b* for the other nerve branch, *b*, and R*ab* for both branches stimulated simultaneously are shown in the fourth and sixth columns respectively; the arithmetical sum of R*a* and R*b*, in the fifth column. The ratio of R*ab* to R*a* + R*b*, denoted as percentage of additivity, is shown in the seventh column. The average of the forty measurements shown in table 2 is 96.0 per cent. The mean variation is 7.48 per cent which may be compared with the mean variation of 5.5 per cent in the control experiment with both muscles shown in table 1.

Discussion. Several factors suggest themselves as sources of error: 1, the stretch of the muscle and tendon during contraction; 2, asynchronism in the contraction of the two portions of the muscle when simultaneously excited; 3, the obliquity of the muscle fibers; 4, the curve indicating the function relating tension and length of the muscle.

The stretch of the muscle and tendon would affect the results only if the relationship between extension and load were not linear. Over any considerable range of loads one would not expect to find the extensibility constant. In experiments performed upon the muscle-tendon of the lateral head of the gastrocnemius of three decerebrate cats, we found an average extension of 0.6 mm. per kgm. over a series of loads ranging from 1.4 kgm. to 10 kgm. Quednau obtained a figure of 0.5 mm. per kgm. for the tendo achillis of the frog. We found in the additivity experiments, that the last increments of load in determining the maximal tension of the muscle may be in the neighborhood of 0.1 kgm. (occasionally more) for about 0.5 mm. on the drum. These increments are made when the total tension has already reached a magnitude of 4 kgm. to 12 kgm. It is quite possible that in this case the extensibility of the muscle-tendon is not linear and may, therefore, affect results. It is certain that the extensibility of the muscle-tendon does allow muscle fibers to contract without registering on the drum, since portions of the muscle may be seen to contract without movement of the lever.

It is possible to obtain considerable differences in the time of contraction of the two portions of the muscle when they are excited simultaneously through the two nerves, *a* and *b*. In a few experiments bearing upon this point we were able to obtain a difference of several hundredths of a second. But such differences in time are not sufficient to explain the marked differences in additivity that have been reported by others or obtained at times by ourselves. The total contraction covers an appreciable interval of time and the time during which the muscle is contracting at its maximum, for any given stimulation and for any given load, is relatively a large proportion of the total duration of the contraction. The degree of asynchronism would have to be much larger than that actually found, in order to make an appreciable difference in the final tension developed.

The obliquity of the muscle fibers with reference to the central tendon has been said to militate against additivity. Exact information is lacking which would permit one to estimate the magnitude of such an influence. Besides the obliquity of the fibers with reference to the central tendon, there are also to be considered the factors of change of angle of the fibers during contraction, the shift of the central tendon during fractionate response, and the relative amounts of innervation by the two nerves of the two groups of oblique muscle fibers which are separated by the central tendon.

The nature of the tension length relationship is fundamentally involved

in the question of the cause of variations in additivity. Arithmetical summation is to be expected only in case the relationship between tension and length is linear. If the curve of this function is not linear in the region concerned in these summation experiments, one could not expect 100 per cent additivity. An accurate determination of this relationship for the muscles used in the additivity experiments would perhaps explain the attainment of additivity to 100 per cent in some cases and not in others.

We are greatly indebted to Dr. G. N. Stewart for inspiration and guidance and to Dr. J. M. Rogoff for direction and many helpful suggestions throughout this investigation.

SUMMARY

1. Forty measurements are reported of the tension developed in the lateral head of the gastrocnemius of decerebrate cats when it is excited by each nerve separately and by both simultaneously. The sum of the fractionate tensions divided into the tension of the whole muscle averages 96 per cent.

2. It is concluded that muscular tension is essentially additive under the conditions investigated.

3. These results support the uni-axonal conception of muscle innervation.

BIBLIOGRAPHY

AGDUHR, E. 1916. Anat. Anzeiger, xlix, 1.
1919. Anat. Anzeiger, lii, 271.
BOYD, M. 1926. This Journal, lxxviii, 254.
CATTELL, McK. 1928. Journ. Physiol., lxvi, 431.
CATTELL, McK. AND P. G. STILES. 1924a. Science, lix, 383.
1924b. This Journal, lxix, 645.
DEBOER, S. 1926. This Journal, lxxv, 285.
EXNER, S. 1885. Pflüger's Arch., xxxvi, 572.
FULTON, J. F. 1926. Muscular contraction and the reflex control of movement. Baltimore, Williams & Wilkins Company.
GAD, J. 1882. Festschrift zur Feier des 300 jahrigen Bestehens der Julius Maximilians, Universität zu Wurzburg.
KARRER, E. AND H. C. STEVENS. 1928a. Journ. Lab. Clin. Med., xiv, 266.
1928b. Journ. Lab. Clin. Med., xiv, 265.
1928c. Journ. Lab. Clin. Med., xiv, 171.
1928d. Science, lxviii, 87.
KATZ, L. N. 1925. Proc. Roy. Soc. B., xcix, 1.
LEDERER, R. AND F. LEMBERGER. 1907. Pflüger's Arch., cxix, 95.
QUEDNAU, W. 1926. Pflüger's Arch., ccxii, 541.
SAMOJLOFF, A. 1924. Pflüger's Arch., cciv, 691.
SAMOJLOFF, A. AND W. WASSILJEWA. 1925. Pflüger's Arch., ccx, 116.
WEED, L. H. 1927. Bull. Johns Hopkins Hosp., xl, 117.

THE TOXIC EFFECTS OF DEFIBRINATED BLOOD WHEN PERFUSED THROUGH THE ISOLATED MAMMALIAN HEART

JULIA HERRICK[1] AND J. MARKOWITZ

From the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minnesota

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There is a growing belief among biologists in general that perfusion experiments are untrustworthy and that data so obtained need bear little relationship to the function of the organ in situ. Such a belief is probably a safe one to adopt, but it is obvious that some functions of an organ or tissue cannot be studied except when they are separated from the organism. The chief objection that can be urged against perfusion experiments is that one has no means of telling whether the perfused organ is in a reasonably physiologic condition or whether it is "dying" rapidly, in which case it would be unwise to accept the data as having much significance. In the case of the heart, this objection loses much of its force; as long as the perfused heart is beating regularly and vigorously the heart may be considered as very much alive, and a study of the metabolism of such a preparation would be a profitable one from the point of view that it represents the metabolism of living mammalian muscle. When one wants to study the metabolism of working muscle, one has available the heart-lung preparation of Starling, in which the amount of work imposed on the heart can easily be kept constant.

In the past most of the studies on perfused hearts have been concerned with problems in circulation, and valuable information has thus been derived. There are two other uses to which perfused hearts may be subjected: they seem to be ideally suited for the study of muscle metabolism in general, and they also serve the important function of acting as test objects for the problem of the factors that determine the survival period of perfused organs. It is a safe assumption that whatever improves the condition of the beating heart will be advantageous to any perfused organ and, on the contrary, whatever harms such a preparation will likewise harm any perfused organ. Such an assumption is obviously arbitrary but we are adopting it because of the great convenience of using the heart as a

[1] Fellow in Biophysics. Section on Physics and Biophysical Research, The Mayo Clinic.

test object. The perfusing fluid for the heart is probably not unique in composition. As is well known, the heart requires a balanced mixture of cations for its proper functioning. However, this is also true of biologic tissues in general, as Loeb has shown. In the case of the heart, this departure from the balance is made manifest immediately. There is much evidence that corresponding disturbances occur in any tissue that is exposed to an unbalanced mixture of cations.

This paper is a record of the unexpected difficulties we have encountered in searching for a good perfusing fluid for the heart.

METHOD. We have found that the most convenient apparatus for perfusing the isolated mammalian heart is that of Locke and Rosenheim (1907). It is superior to Gunn's apparatus, which, although capable of permitting graphic records of the heart beat, possesses the disadvantage of not being automatic, requiring a manual return of the perfusing fluid to the reservoir. We have supported the apparatus on pipe tubing, bolstered it suitably, and have slightly modified it so that graphic tracings of the heart beat may be taken. Just before the aorta is tied on the cannula a needle containing silk is threaded through the apex and tied. The other end of the thread is passed through the narrow aperture of the glass tubing at the bottom of the heart chamber and tied to the heart lever. When a tracing is not being taken the constant drip along the thread is abolished by plugging the bottom of the tube with a wooden swab stick.

Rabbits were generally used in the investigation, although the early experiments were performed on dogs. The technic of setting up the heart in the perfusion apparatus was as follows: The rabbit was anesthesized with ether and a cannula was inserted into the carotid artery. About 15 cc. of blood were then withdrawn into a beaker, and the blood was defibrinated by whipping it gently with wooden swab sticks. In the meantime about 20 cc. of Locke's solution were slowly injected into the animal through the cannula in the carotid. The process was repeated several times until the blood was quite watery. The mixture of blood and Locke's solution was defibrinated for another ten minutes and carefully strained through gauze. This was added to the reservoir of the perfusing apparatus and permitted to flow into the worm. The heart was gently excised from the thorax, care being taken to avoid any injury to the auricles and to leave a long strip of the aorta. It was placed in a beaker of cold Locke's solution and gently kneaded to wash the blood out of its chambers. The tip of a 50 cc. syringe containing Locke's solution was then inserted into the aorta and the blood clot washed out by a forcible jet or two. The thumb and fore-finger grasped the aorta firmly about the nozzle of the syringe, and the coronary arteries were gently perfused with about 50 cc. of the solution, or until the heart was quite bloodless. The nozzle of the syringe was introduced into the caval and pulmonary orifices respec-

tively and the chambers were completely washed of blood. The heart was transferred to the perfusion apparatus: with the screw-clip above the cannula in the aorta so adjusted as to result in a constant drip of perfusion

Fig. 1

Fig. 2

Fig. 1. Heart beating in Locke's solution; control experiment. Tracing *1* at 10:15 a.m.; tracing *2* at 10:30; subsequent tracings at half-hour intervals, ending at 1:00 p.m.

Fig. 2. Defibrinated blood added to perfusing fluid immediately after defibrination. Tracing *1*, heart beating in Locke's solution; tracing *2*, five minutes after tracing *1*. Tracings *3* to *12* were made after the addition of defibrinated blood: tracing *3*, two minutes after; tracing *4*, seven minutes after; tracing *5*, twelve minutes after; tracing *6*, seventeen minutes after; tracing *7*, twenty-two minutes after; tracing *8*, twenty-seven minutes after (here and subsequently the heart shows varying degrees of block); tracing *9*, thirty-seven minutes after; tracing *10*, forty-seven minutes after; tracing *11*, sixty-seven minutes after, and tracing *12* ninety-seven minutes after.

fluid, the aorta was filled with fluid and the cannula was slipped into the aorta. It is essential that air embolism of the coronaries be guarded against, and for the next ten minutes the heart has to be carefully watched for bubbles which may work their way into the aortic cannula from the

left ventricle. When bubbles appeared they were instantly expelled by opening the clip in the side arm of the cannula in the aorta.

RESULTS. With the foregoing technic we have tested the value of Locke's solution, of defibrinated blood and of heparinized blood from the same animal with regard to the value of these as perfusing fluids. We can testify to the importance of making Locke's solution from glass-distilled water and absolutely pure chemicals. Especial care was taken to avoid all possible contamination of perfusing fluid with traces of heavy metal.

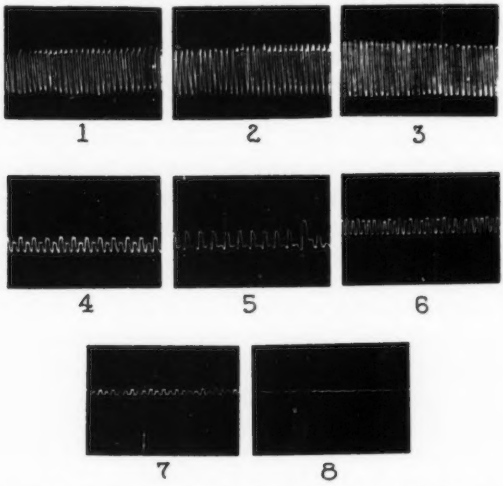

Fig. 3. Fifty cubic centimeters of defibrinated blood which had stood on ice four and a half hours added to 250 cc. Locke's solution, after bringing it to body temperature. Tracing *1*, heart beating in Locke's solution; tracing *2*, five minutes after tracing *1*. Tracings *3* to *8* were made after the addition of defibrinated blood: tracing *3*, two minutes after; tracing *4*, seven minutes after; tracing *5*, twelve minutes after (the heart shows a 2:1 block); tracing *6*, twenty-two minutes after; tracing *7*, forty-two minutes after, and tracing *8*, seventy-two minutes after.

Confirming the work of Locke and Rosenheim, we found it easily possible to keep a perfused heart alive for 8 to 10 hours with pure oxygenated Locke's solution (fig. 1).

In each case the experiment was terminated because of edema of the heart which embarrassed it mechanically. There was never any sign of irregularity when the experiment was terminated.

It was expected that the use of defibrinated blood would constitute an improvement in this technic, but the results were uniformly constant in that such is absolutely not the case. Within about an hour after the

beginning of the perfusion, the flow through the coronary arteries was obviously curtailed and the heart manifested various irregularities. The coronary arteries appeared intensely constricted on inspection. Eventually the flow through the coronaries was so slight that the blood which reached the heart was practically at room temperature, as shown by the temperature of the heart chamber. The heart rate became progressively slower during the experiment largely, if not entirely, on account of the decrease in temperature.

This toxicity of defibrinated blood for the perfused heart was easily verified by adding the animal's own defibrinated blood to a heart that was beating well while perfused with Locke's solution. The effects here were invariably very striking within a few minutes (figs. 2 and 3).

In two other experiments we used heparinized blood as the perfusing fluid. Fifty milligrams of heparin were injected intravenously into a rabbit. A cannula was inserted into the carotid artery and about 20 cc. of blood were repeatedly removed, with injections of Locke's solution between bleedings. About 150 cc. of diluted blood were thus obtained. To this were added 100 mgm. of heparin dissolved in a few cubic centimeters of Locke's solution. The heart beat splendidly with this perfusion fluid for not quite an hour, when the beat suddenly became irregular. The blood on examination was full of fine stringy clots, some of which had evidently formed below the glass-wool trap, causing coronary embolism. Another such experiment performed on a dog's heart gave similar results.

COMMENT. Although the vasoconstrictor property of shed blood has been known for years, it is only recently, as a result of the experiments of Starling and his pupils, that the intensely vasoconstricting power of defibrinated blood has been properly appreciated. In the past this strikingly poisonous property of defibrinated blood for the perfused heart has not been emphasized, apparently for two reasons: the blood was often diluted with a very large volume of physiologic solution of sodium chloride, and it was not known until the work of Locke and Rosenheim that a perfused heart was capable of surviving for an exceptionally long period.

Working with the heart-lung-kidney circulation, Starling and Verney (1925) noted that defibrinated blood was intensely vasoconstricting to the kidney unless this blood were perfused several times through the lungs before the kidney was admitted into the circulation. We have in this laboratory seen the same phenomenon; in a heart-lung perfusion defibrinated blood is not manifestly toxic to the heart. Its intense vasoconstricting property is evident when it is circulated through the liver or kidney before it has been perfused through the lungs for several minutes. It is almost impossible to force more than a few cubic centimeters of blood a minute through the kidney in a heart-lung-kidney perfusion except when the blood has previously been circulated through the lungs. Evidently the lung removes or destroys the toxin of defibrinated blood.

Anrep and Stacey (1927) studied the coronary blood flow in a heart-lung circulation by inserting a cannula into the coronary sinus, measuring blood flow by means of the hot wire anemometer. Among their interesting observations they mentioned that during the course of the experiment the coronary arteries gradually expanded. We would suggest as the explanation for this phenomenon the gradual removal by the lungs of the remaining traces of vasoconstrictin.

Laboratory manuals of physiology and pharmacology recommend that for heart perfusion experiments the animal's blood be defibrinated and added to Locke's solution. Our experiments show definitely that this is not the best way to perfuse a heart. The best and most convenient perfusing fluid for the heart in a laboratory experiment is oxygenated Locke's solution made up according to Locke and Rosenheim's specifications (1907). There is, of course, no object in perfusing the isolated heart by first passing the blood through the lungs before admitting it into the cannula in the aorta. An easier technic for this purpose is the heart-lung circulation of Starling. We have seen such hearts in good condition eight hours after the experiment was begun and the experiments in each case were terminated because of extensive edema of the lungs. In this stage ventilation of the lungs, to be effective, has to be carried on with pure oxygen.

The poisonous property of defibrinated blood is not entirely due to its vasoconstricting power. In one experiment we added amyl nitrite to the perfusing fluid, and although improvement in coronary blood flow resulted the heart was not definitely improved.

With regard to the nature of the vasoconstrictin in defibrinated blood, one might state definitely that it is not epinephrin for two reasons: the perfused heart gives no such reaction as we have been getting to epinephrin, and defibrinated blood on injection intravenously, even in remarkably small quantities, causes a pronounced fall in blood pressure accompanied by expansion of the spleen and diminution in size of the kidney. This observation, which was originally made by Brodie (1900), has been repeatedly confirmed by us. Working with the excised ox carotid in Locke's solution Janeway, Richardson and Park (1918) made some very careful observations on the vasoconstrictor action of defibrinated blood and of blood serum. They decided that this was due to a crystalloid that was insoluble in ether and chloroform, and that while it was present in coagulated blood, it was not dependent for its formation on the actual formation of a blood clot. They found an extract of blood platelets to have a marked vasoconstrictor action. This was not the case for extracts of leukocytes and erythrocytes.

Hirose (1918) showed that the vasoconstrictor property of shed blood paralleled the platelet count of the circulating blood. Defibrinated blood

with platelet counts far below normal developed little, if any, power of vasoconstriction.

We consider the newer work on vasoconstrictins in blood to have great possibilities as regards certain puzzling phenomena in biology. To one who has read the paper of Dale and Kellaway (1922) on anaphylatoxins, the suspicion becomes a strong one that most of the so-called anaphylatoxins are none other than this vasoconstrictin in shed blood whose source most probably is disruption of blood platelets.

The histamine theory of shock, on the whole, has been a sterile hypothesis as regards the actual demonstration that surgical shock is concerned with a histamine mechanism. For instance, we wish to point out that histamine in a rabbit always causes a rise in blood pressure, and yet the rabbit is very prone to surgical shock. It seems just possible to us that the vasoconstrictins of defibrinated blood may play a part in the production of surgical shock. Those factors that are most conducive to the production of shock are just those factors that would bring about trauma to a large volume of blood. We shall have more to say about this in the future.

Vasoconstrictin may be concerned with yet another phenomenon, namely, the production of arteriosclerosis as the animal becomes "old." It is barely possible that the constant production of small quantities of this substance in the animal body is responsible for this condition, for in vasoconstrictin we possess a substance that is, on the one hand, probably continuously produced in the animal body and, on the other hand, is actively poisonous to the smooth muscle of blood vessels.

We wish to point out what, perhaps, has previously not been emphasized, that normal blood is potentially actively poisonous. The mechanism of certain overwhelming infections may be concerned with a change in the blood, and it seems a tenable hypothesis to us that surgical shock may be due to a similar change.

SUMMARY

It has been shown that whereas the mammalian heart will beat for hours when perfused with pure oxygenated Locke's solution, the addition of the animal's defibrinated blood to the perfusion fluid is actively poisonous to the heart. The defibrinated blood may be detoxified by repeated passage through the lungs (confirming the work of Starling and Verney). These observations were brought into line with what has previously been found by others in regard to certain poisonous properties of shed blood. The substance responsible for this action on the heart has been found to cause a marked prolonged drop in blood pressure. Several theoretic considerations are raised about the possible rôle of this substance in biologic phenomena.

BIBLIOGRAPHY

Anrep, G. V. and R. S. Stacey. 1927. Journ. Physiol., lxiv, 187.
Brodie, T. G. 1900. Journ. Physiol., xxvi, 48.
Dale, H. H. and C. H. Kellaway. 1922. Phil. Trans. Roy. Soc. London, ccxi, ser. B., 273.
Hirose, K. 1918. Arch. Int. Med., xxi, 604.
Janeway, T. C., H. B. Richardson and E. A. Park. 1918. Arch. Int. Med., xxi, 565.
Locke, F. S. and O. Rosenheim. 1907. Journ. Physiol., xxxvi, 205.
Starling, E. H. and E. B. Verney. 1924–1925. Proc. Roy. Soc. London, xcvii, Ser. B, 321.

THE EFFECTS OF IRRADIATED DEFIBRINATED BLOOD ON THE PERFUSED HEART[1]

JULIA F. HERRICK[2]

From The Mayo Foundation, Rochester, Minnesota

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Because of the various facts accumulated by different investigators, among them Laurens (1928), Mayer (1926), Mayerson and Laurens (1928) and Reed and his co-workers (1923–1928) regarding the effects of ultraviolet irradiation on the circulation of the blood, it seemed that it might be of value to study the action of an isolated mammalian heart (rabbit) when perfused with irradiated blood.

In the first method a quartz tube or chamber was inserted immediately above the heart chamber of the perfusion apparatus. This allowed maximal energy from the air-cooled quartz mercury vapor lamp (Victor X-ray Corporation) to be incident on the blood. Special precautions were taken to filter out as completely as possible any heat waves from the lamp by using a water cell 2.5 cm. thick with two large quartz windows. This method had to be abandoned because the heart could not be kept alive sufficiently long to detect changes due to the irradiation. It was necessary, therefore, to develop a technic for perfusion of the heart which would enable this organ to be kept alive for five or more hours. This technic is described fully in a previous article (Herrick and Markowitz, 1929).

Heparinized blood could not be used in these experiments on account of clotting. Therefore defibrinated blood, although toxic, was employed in order to determine whether or not irradiation of the blood produced any effect on the action of the heart. The following procedures on two rabbits were carried out.

The first rabbit was bled and its blood irradiated for four and a half hours. Especial precautions were taken to protect the blood from the heat emitted by the quartz mercury vapor lamp. The blood was placed in a large Petri dish so as to expose the maximal surface to the radiations from the lamp. The dish was placed on finely chopped ice and covered with a water filter. The mercury arc was about 17.5 cm. from the blood

[1] Work done in the Section on Physics and Biophysical Research, The Mayo Clinic and the Division of Experimental Surgery and Pathology, The Mayo Foundation.

[2] Fellow in Biophysics.

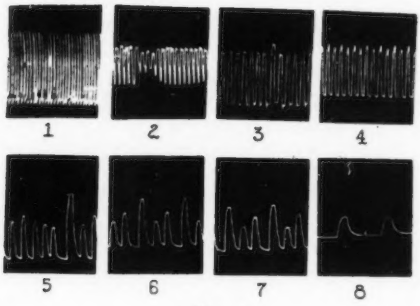

Fig. 1

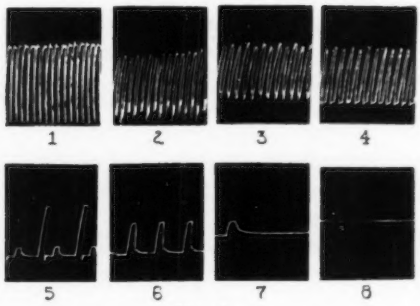

Fig. 2

Fig. 1. Irradiated blood added to perfusing fluid. Curve *1*, Ringer-Locke's solution only; *2*, five minutes after adding irradiated defibrinated blood; *3*, ten minutes later; *4*, thirty minutes later; *5*, thirty minutes later, a 2:1 heart block; *6*, ten minutes later; *7*, fifteen minutes later; *8*, thirty-five minutes later.

Fig. 2. Irradiated blood added to perfusing fluid. Curve *1*, Ringer-Locke's solution only; *2*, fifteen minutes later; *3*, five minutes after adding irradiated defibrinated blood; *4*, twenty-five minutes later; *5*, thirty minutes later, 2:1 heart block; *6*, thirty minutes later; *7*, sixty minutes later; *8*, fifteen minutes later.

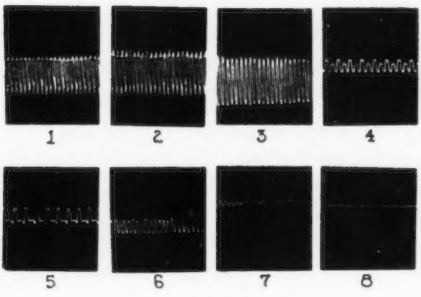

Fig. 3

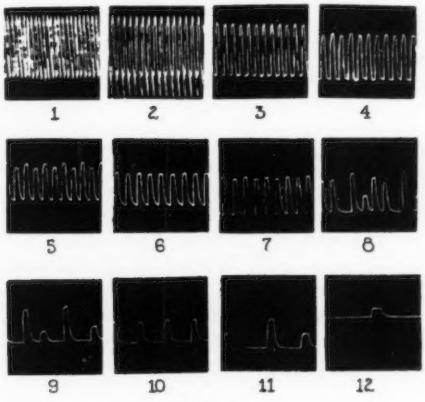

Fig. 4

Fig. 3. Defibrinated blood which has stood on chopped ice for four and a half hours before addition to perfusing fluid. Curve *1*, Ringer-Locke's solution only; *2*, five minutes later; *3*, two minutes after adding cooled defibrinated blood; *4*, five minutes later; *5*, five minutes later, 2:1 heart block; *6*, ten minutes later; *7*, twenty minutes later; *8*, thirty minutes later.

Fig. 4. Defibrinated blood which has not been cooled added to perfusing fluid. Curve *1*, Ringer-Locke's solution only; *2*, five minutes later; *3*, two minutes after adding defibrinated blood; *4*, five minutes later; *5*, five minutes later; *6*, five minutes later; *7*, five minutes later; *8*, five minutes later, a 2:1 heart block; *9*, ten minutes later; *10*, ten minutes later; *11*, twenty minutes later; *12*, thirty minutes later.

and the lamp was operated at 90 volts. The blood became dark and had a peculiar odor; it contained large clumps that had to be filtered off before the perfusing medium was added.

The second rabbit was used for the perfusion proper. Shortly before the end of the period of irradiation the heart perfusion was started, Ringer-Locke's solution being used for the perfusing fluid. The irradiated blood was added after the beat of the heart had become regular and vigorous. Figures 1 and 2 show the changes in heart beat due to irradiated defibrinated blood.

A similar experiment was performed in which defibrinated blood, which had not been irradiated but had been allowed to stand on finely chopped ice for four and a half hours, was added to the perfusing fluid (fig. 3).

Figure 4 shows kymographic records obtained with defibrinated blood kept at the temperature of the room before its introduction into the circulating fluid.

A comparison of the results obtained with irradiated (figs. 1 and 2) and non-irradiated (figs. 3 and 4) defibrinated blood did not show marked differences. Therefore, irradiation of defibrinated blood, per se, does not produce physiologic effects on the action of the perfused heart. Possibly the true effects which might be produced by irradiation are masked by toxic effects of the defibrinated blood. These experiments furnish little if any evidence that changes in the circulation, which have been reported by others as due to irradiation, are of cardiac origin.

BIBLIOGRAPHY

FALK, I. S. AND C. I. REED. 1925–1926. This Journal, lxxv, 616.
HERRICK, J. F. AND J. MARKOWITZ. 1929. This Journal, lxxxviii, 698.
KOCH, F. C. AND C. I. REED. 1925–1926. This Journal, lxxv, 351.
LAURENS, H. 1928. Physiol. Rev., viii, 1.
MAYER, E. Clinical application of sunlight and artificial radiation. Baltimore, The Williams & Wilkins Company, 1926, 468 pp.
MAYERSON, H. S. AND H. LAURENS. 1928. This Journal, lxxxiv, 1.
REED, C. I. 1923. This Journal, lxv, 477.
1925. Ibid., lxxiv, 511, 518, 525.
1927. Arch. Physical Therap., viii, 108.
1928. Science, lxviii, 203.
REED, C. I. AND W. R. TWEEDY. 1926. This Journal, lxxvi, 54.

THE CO_2 ABSORPTION CURVE AND BUFFER VALUE OF THE BLOOD IN PHYSICAL HYPERTHERMIA[1]

M. GARCIA BANUS

IN COLLABORATION WITH J. M. WILCOX

From the Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio

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Many factors, aside from the increase in temperature, affect the acid-base equilibrium of the blood in fever; particularly the causative infection which either produces toxins or initiates the destruction of certain tissues, including the blood itself. Two methods have been adopted by investigators in order to reduce the number of variables and limit the problem to the effect of temperature alone: 1, the effects of temperature on the physico-chemical constants which determine the acid-base equilibrium of the blood have been studied *in vitro*; and 2, the changes in the blood from animals and human beings whose temperature had been elevated by physical means have been investigated.

The former method was employed, among others, by Stadie and Martin (1924) and Stadie, Austin and Robinson (1924). By taking into account the changing values of the constants it is possible from data obtained at normal temperature to calculate the condition of the blood at fever levels. These calculations, however, do not consider the possibility of other changes occurring in the blood when it is circulating in an animal whose temperature is higher than normal for a considerable period of time.

The second method, which has been followed by Haggard (1920), Cajori, Crouter and Pemberton (1923), Adolph (1924), Landis et al. (1925) and Cheer (1928), has definitely shown that short periods of hyperthermia (less than half an hour of actual high body temperature) cause a lowering of the CO_2 tension and content, and an increase in the pH of the blood. The effect of such short periods of hyperthermia, which practically amount to the simple effect of a change in temperature, are probably not comparable to the effect of a more protracted hyperthermia, such as occurs in fever. The possibility that the high temperature if maintained for longer periods may lead to further changes must be investigated. That such further changes occur may be suspected from the observations of Flinn and Scott (1923)

[1] A preliminary report of this paper has already been published in This Journal, 1928, lxxxv, 348.

who found a decrease in the alkaline reserve when dogs were subjected to more prolonged hyperthermia (about two hours). It is therefore desirable to study more extensively the character of the acid-base changes in the blood of animals subjected for longer periods to high body temperature induced by physical means.

This was not, however, the chief cause of our reinvestigation of the subject; it was rather our aim to obtain further information of the factors responsible for the changes that occur during prolonged hyperthermia. For this purpose we made a careful study of the CO_2 absorption curve and of the buffer value of whole blood during hyperthermia lasting for more than two hours. The CO_2 absorption curve of the blood depends on two of the factors which determine its acid-base equilibrium, i.e., on the alkaline reserve which determines the relative height of the curve, and on the buffer value which determines its slope. The latter can be more distinctly shown plotting the curve logarithmically as was done by Peters (1925).

The interrelation of these factors during hyperthermia has not been previously attempted. Only Adolph (1924) actually determined the CO_2 absorption curve in two cases. As far as we know the buffer values have not been studied. In regard to changes in alkaline reserve the evidence is contradictory. Adolph found no change in human subjects; Cajori, Crouter and Pemberton (1923) observed an increase in five out of ten cases; while Flinn and Scott (1923) noted a decrease in dogs subjected to hyperthermia for about two hours.

In the study of the effect of high body temperatures on the CO_2 absorption curve of the blood and on its buffer value, it is important to remember that the physico-chemical constants of the blood are themselves affected by temperature. Such effects can be ruled out by analyzing and comparing, at the same temperature, the blood obtained from the animal at normal temperature and during hyperthermia. This was accomplished by analyzing all the blood samples obtained by us at 25°C.

Procedure. Dogs, 10 to 15 kilo in weight, were anesthetized by an intraperitoneal injection of chloretone in oil (0.3 gm. per kilo). Alveolar air was drawn by means of a catheter inserted through a tracheal cannula (Banus, 1926). Blood was drawn from a cannula inserted in a carotid artery. Body temperature was registered on a thermometer, the bulb of which lay in the upper esophagus. To raise the body temperature, the dog was immersed up to the neck in a water bath kept within 1°C. by means of a thermo-regulator and stirring device. Experience showed it advisable not to exceed a bath temperature of 42°C. as otherwise the body temperature exceeds 41°C., and the animal expires in less than 6 to 8 hours. Placed in a bath at such a temperature, the dog reaches a body temperature of about 39°C. in 30 to 50 minutes. This moment was considered as the beginning of the hyperthermia periods. The dog reached a body tempera-

ture of 40 to 41°C. in $1\frac{1}{2}$ to $1\frac{3}{4}$ hours, which was maintained throughout the experiment, i.e., often for 8 hours. The fact that it is less by 1 to 2°C. than that of the bath is evidence that we are dealing with physical hyperthermia and not heat fever.

In order to rule out the possible effects of anesthesia, operation, withdrawal of blood, etc., control observations were made under exactly the same experimental conditions, except that the animals were kept at 37°C. for equivalent periods of time.

At suitable intervals, 40 to 50 cc. of blood were drawn from the carotid artery, part of it under oil for the determination of actual CO_2 and oxygen content. Clotting was prevented by the addition of 2 drops of a 2 per cent heparin solution to each 5 cc. of blood. At the same time alveolar air was sampled and analyzed to determine its CO_2 tension.

Three samples were taken at the following times:

Sample I: Before immersion in the bath,—normal blood sample.
Sample II: After two hours of hyperthermia above 39°C.
Sample III: After six hours of hyperthermia above 39°C.

Each sample was analyzed to determine the following characteristics: the CO_2 content; the CO_2 absorption curve or $\frac{\Delta[CO_2]}{\Delta pCO_2}$ curve; the actual pH at alveolar pCO_2; the $\frac{\Delta pH}{\Delta pCO_2}$ curve; the concentration of the blood in total solids; and, in later experiments, the actual oxygen content and the hemoglobin concentration calculated from the oxygen capacity.

The CO_2 and O_2 content of the arterial blood drawn under oil was determined within half an hour after sampling by means of the constant volume apparatus of Van Slyke and Neal.

The pH was determined electrometrically on whole reduced blood at different tensions of CO_2. Clark electrodes, Leeds and Northrup type K potentiometer, and a saturated calomel cell were used. The value of the calomel cell was determined by standardization against N/10 HCl in the usual manner. For the pH determination part of the sample was simultaneously reduced and equilibrated with CO_2 at the desired CO_2 tension in an oscillating tonometer. This was accomplished by admitting a continuous stream of a mixture containing hydrogen and CO_2 saturated with water vapor. Such a mixture was obtained by the method described elsewhere (Banus). The blood was then transferred to the electrode vessels, which were likewise filled with the identical mixture of hydrogen and CO_2. The equilibration and electrometric determinations were in all cases carried out at 25°C., the system being completely enclosed in a carefully controlled thermostat (temperature variation ± 0.5°C.). No change in the concentration of the blood takes place during the successive equilibrations as checked

by actual determinations. Thus, in experiment 21, the concentration in total solids in two samples was 19.7 per cent and 20.3 per cent respectively before equilibration, and 19.7 per cent and 20.25 per cent after equilibration with three different gas mixtures. Simultaneous double determinations of the pH were made in each reading. Experimental checks showed that the error in obtaining the CO_2 tension of the mixture of hydrogen and CO_2 and was less than ± 1 mm. Hg, and that the pH determinations with this method were accurate to within ± 0.01 of a pH.

The pH of reduced arterial blood was determined for each sample at the alveolar CO_2 tension existing at the moment of sampling. Besides this determination the pH at two or three other pCO_2[2] values was obtained thus giving 3 or 4 points on the $\frac{\Delta pH}{\Delta pCO_2}$ curve of each sample.

The CO_2 absorption curve or $\frac{\Delta[CO_2]}{\Delta pCO_2}$ curve was obtained by determining the CO_2 capacity of each sample at 3 or 4 different pCO_2, thus also giving three to four points on the curve. The blood sample was placed in a tonometer within the thermostat at 25°C. and equilibrated with a stream of the desired mixture of air and CO_2. The CO_2 capacity at each pCO_2 was determined in the Van Slyke-Neal constant volume apparatus, two check determinations being made for each value. The error in the determination was never greater than ± 0.2 volume per cent of CO_2. Again no change in the concentration of the blood could be detected after the triple equilibration.

The concentration of the blood was determined as the per cent of total solids according to the method described by Van Slyke. The error of the method, as used, being on the average ± 0.13 in the per cent of total solids (maximum error, ± 0.36).

The hemoglobin content of the blood was calculated from the oxygen capacity of the blood at 25°C., as determined in the Van Slyke-Neal apparatus after saturation with room air. The hemoglobin content obtained by dividing the oxygen capacity by 1.34, is given in grams per 100 cc. of blood. The error of the determination was about ± 0.1 volume per cent of oxygen or less than ± 0.1 gram per cent of hemoglobin.

Results. The data and calculations included in the study of the CO_2 absorption curves of the blood before and after hyperthermia are shown in table 1. To avoid complexity of tabulation only two sets of values for the CO_2 capacity and pH at two pCO_2, viz., at 15 and 45 mm. Hg, are given in columns A and D. These are given both for the normal blood and for the sample in which the effect of hyperthermia was at its maximum. The increase in their values between these two pCO_2, the $\Delta[CO_2]_{45-15\,mm.}$ and

[2] pCO_2 is used in the usual sense, viz., partial pressure of CO_2.

the $\Delta pH_{45-15\ mm.}$, are given in columns B and E respectively. The increment in CO_2 capacity (column B) is given both in volumes per cent and in millimols. From the increment of CO_2 capacity in millimols and the corresponding negative increment of pH (column E) the buffer value of the blood was calculated. This is entered in column G. This buffer value is not given in terms of the increase of bicarbonate concentration as suggested by Van Slyke in his later papers, but is calculated from the total CO_2

TABLE 1

Effect of hyperthermia on CO_2 absorption and buffer values of the blood

EXPERIMENT NUMBER	HOURS OF HYPERTHERMIA	(A) CO_2 CAPACITY IN VOLUMES PER CENT AT pCO_2		(B) $\Delta[CO_2]_{45-15}$		(C) CHANGE IN THE $\Delta[CO_2]_{45-15}$		(D) pH AT pCO_2		(E) $-\Delta pH_{45-15}$	(F) CHANGE IN THE ΔpH_{45-15}	(G) $\frac{\Delta[CO_2]}{-\Delta pH}$	(H) ACTUAL pH AT ALVEOLAR pCO_2
		15 mm.	45 mm.	Volumes per cent	Millimols	Volumes per cent	Millimols	15 mm.	45 mm.				
5	Before	28	40	12	5.4			7.58	7.21	0.37		14.6	7.31
	6 hours	22	58	26	11.7	+24	+6.3	7.55	7.27	0.28	−0.09	41.8	7.61
6	Before	33	44	11	4.95			7.52	7.19	0.33		15.0	7.26
	6 hours	26	39	13	5.8	+2	+0.9	7.45	7.16	0.29	−0.04	20.0	7.45
7	Before	31	40	9	4.05			7.56	7.23	0.33		12.3	7.26
	6 hours	26	47	21	9.4	+12	+5.3	7.46	7.22	0.24	−0.09	39.0	7.49
9	Before	36	45	9	4.05			7.46	7.15	0.31		13.0	7.22
	6 hours	28	47	19	8.5	+10	+4.4	7.37	7.14	0.23	−0.08	37.0	7.49
20	Before	34	47	13	5.8			7.59	7.24	0.35		16.0	7.24
	2 hours	27	55	28	12.6	+15	+5.8	7.57	7.29	0.28	−0.07	45.0	7.28
21	Before	25	37	12	5.4			—	—	—		—	7.31
	2 hours	21	35	21	9.4	+9	+4.0	—	—	—	—	—	7.33
22	Before	28	51	23	10.35			7.49	7.12	0.37		28.0	7.22
	2 hours	21	52	31	13.9	+8	+3.5	7.51	7.17	0.34	−0.03	41.0	7.45
Maximum errors.		±0.5		±1.0	±0.45	±1.0	±0.45	±0.01		±0.02	±0.02	±2.0	±0.01

change according to his earlier formula, i.e., buffer value $= \frac{\Delta[CO_2]}{\Delta pH}$. This was done for the following reasons: As Peters, Bulger and Eisemann (1924) pointed out, the calculation of the bicarbonate concentration requires an accurate knowledge of the solubility of CO_2 in whole blood, which depends on the hemolgobin concentration; for this reason they consider the calculation of the buffer value from the total CO_2 change as more reliable for whole blood. Furthermore, the hemoglobin concentration was very variable in our experiments and was not determined in all cases. The earlier formula of Van Slyke's is certainly better suited to our experiments, and gives as

clear an idea of the buffer value and its changes, as the formu a based on the bicarbonate concentration. The pH values are those of completely reduced blood, the only value that could be obtained directly with our method. This of course makes all the buffer values smaller than those of oxygenated

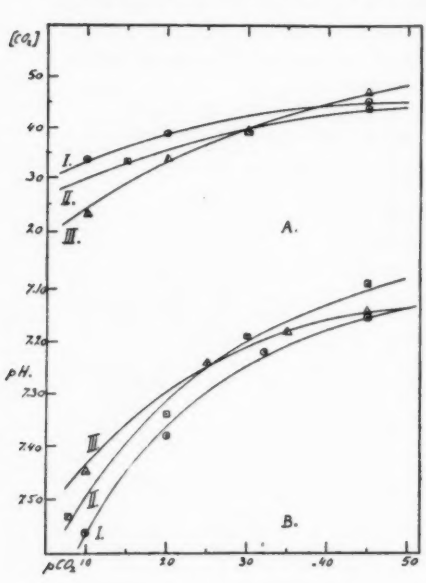

Fig. 1

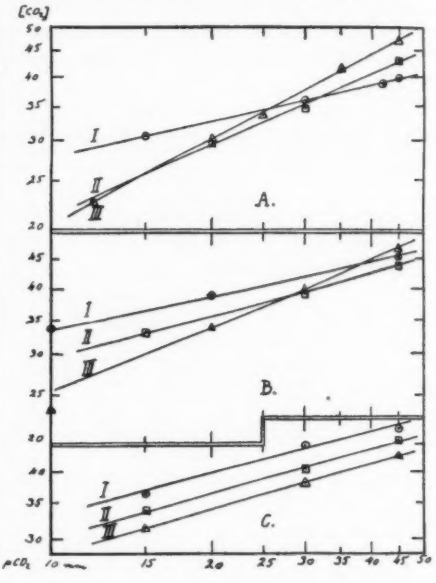

Fig. 2

Fig. 1. Curves plotted from data obtained in a typical experiment (expt. 9). A: The CO_2 absorption curves or $\frac{\Delta[CO_2]}{\Delta pCO_2}$ curves; B: The $\frac{\Delta pH}{\Delta pCO_2}$ curves. In both groups of curves: I, ⊙—⊙: curve of normal blood. II, ⊡—⊡: curve of blood after 2 hours of hyperthermia above 39°C. III, △—△: curve of blood after 6 hours of hyperthermia.

Fig. 2. The CO_2 absorption curves plotted logarithmically for two typical experiments, A: Experiment 7, B: Experiment 9; as compared with those of a control experiment, C: Experiment 17. I, ⊙—⊙: curve of normal blood or blood after ¾ hour anesthesia. II, ⊡—⊡: curve of blood after 2 hours of hyperthermia above 39°C. or 4¾ hours of anesthesia. III, △—△: curve of blood after 6 hours of hyperthermia or 8¾ hours of anesthesia.

blood. The difference between the pH of oxygenated blood and that of reduced blood, at the same pCO_2 depends on the amount of hemoglobin present. But even when the hemoglobin changes as much as 30 per cent, the error, according to L. J. Henderson's nomogram (Henderson et al., 1924), is about pH 0.02, which is practically within our limits of error.

The CO_2 absorption curves, as well as the $\frac{\Delta pH}{\Delta pCO_2}$ curves, of all the experiments were plotted in a manner similar to that of the typical experiment illustrated in figure 1. As will be noted, three or four points, corresponding to a similar number of observations on each sample were made use of. The CO_2 absorption curves were also plotted logarithmically, as illustrated by two typical experiments in figure 2, A and B. The values for all the other experiments whose curves are not given can be studied from the data in table 1.

A glance at the group of curves in figure 1, A, representing the CO_2 absorption curves under normal conditions (curve I) and after 2 and 6 hours of hyperthermia, respectively (curves II and III), shows that significant changes have taken place. While curve II occupies a lower position at all pCO_2, curve III does so only at low tensions, it crosses the normal curve, and actually reaches a higher position at 45 mm. pCO_2. One cannot speak of a rise or a lowering of such a curve, i.e., of an increase nor of a decrease of the alkaline reserve on which the relative position of the curve depends; while the CO_2 capacity is always lower than normal at low CO_2 tensions, it is as large or even larger than normal at higher CO_2 tensions (see column A, table 1). In the case illustrated in figure 1, the CO_2 capacity at 40 mm. pCO, which is usually considered as the measure of the alkaline reserve, is almost the same as in the normal blood, and nevertheless the condition of the blood is very much altered.

Such a change in the character of the CO_2 absorption curve cannot be attributed to experimental error. In the first place, similar points plotted as a logarithmic curve fall very nearly in a straight line. Furthermore, the change in slope was observed in all our experiments, the only difference being its degree, the duration of hyperthemia required for its appearance, and the duration of the effect. In many cases it appeared as early as 2 to 3 hours after the onset of hyperthermia (expts. 5, 6, 7, 19, 20, 21, 22), but was more clearly marked after 6 hours (expts. 5, 6, 7). This is well shown by the crossing of both hyperthermic curves in figure 2, A. In experiments 8 and 9 it was not clearly established until the sixth hour. In some cases (expts. 20 to 22) the maximum change in the slope of the curve occurred at the end of 2 hours, and the curve dropped to a very low level after 6 hours of hyperthermia. In these cases its slope became normal again or even less than normal. This can be attributed to an excessive production of fixed acids which occurred in these particular cases. For instance, the actual pH of the blood in experiment 22 after two hours of hyperthermia was 7.52 with an alveolar pCO_2 of 15 mm., while after 6 hours it was 7.44 with a lower pCO_2, namely, 12 mm.

The low position of the points at small CO_2 tensions indicates a certain increase in the amount of fixed acids combined with the normal base of the

blood. This is confirmed by the fact that the pH at this pCO_2 is always lower than in the normal blood (column D, table 1). But at higher pCO_2, the CO_2 capacity is not correspondingly low; it is normal or even higher. This makes the increment of the CO_2 content between two points (the $\Delta[CO_2]$ between 15 and 45 mm. pCO_2, column B) greater in the blood of hyperthermia cases than in the normal blood. As the CO_2 tension increases hyperthermic blood must liberate an amount of base in excess of that set free by the normal. This excess can be calculated from the difference in the increment of CO_2 between the hyperthermic and normal blood which amounts in all cases but one (where it is very small) to from 8 to 24 volumes per cent of CO_2 or 3.5 to 6.3 millimols of CO_2. The latter figures, given in column C, actually represent the amount of base liberated by the blood in excess of the normal as the pCO_2 changes from 15 to 45 mm. This is true because the figures are obtained by subtracting two values, each including the amount of CO_2 dissolved by the blood at the same pCO_2.

It can be predicted from the excess of base liberated by the hyperthermic blood that the increased slope of the CO_2 absorption curve is accompanied by a decrease in the slope of the $\frac{\Delta pH}{\Delta pCO_2}$ curve. Such was the case in all our experiments as is clearly revealed by consulting the data in column D, table 1, or the curves of a specific experiment plotted in figure 1, B. It will be observed that the blood is more acid in the hyperthermia sample at low pCO_2 (all cases except expt. 22), but that it has almost the same pH or is more alkaline than the normal at high pCO_2. Consequently, the change in pH between pCO_2 15 to 45 mm. is less in hyperthermic than in normal blood (column E, table 1).

The figures given in columns B and E for comparable determinations show that the ratio $\frac{\Delta[CO_2]}{\Delta pH}$, which determines the buffer value of the blood, has a larger numerator and a smaller denominator in hyperthermic than in normal blood. It follows that the buffer value of the blood must increase as an effect of hyperthermia. This increase in the buffer value can also be deducted from a mere inspection of the logarithmic CO_2 absorption curves (e.g., fig. 2, A and B), for Peters, Bulger and Eisenmann (1924) have shown that the slope of this curve depends on the buffer value of the blood. The actual values given in column G of table 1 show that a definite and very marked increase occurs, not only when the original buffer value was low, but also when, as in experiment 22, the normal buffer value of the blood was fairly high.

We must now evaluate the significance of these results. In the first place, the possible effect of the anesthetic used must be taken into consideration. To eliminate this factor a series of control experiments was made. The animal was maintained under the same experimental conditions at

37°C. Samples were taken immediately after anesthesia was effective and then at the following times: 2 to $4\frac{3}{4}$ hours, and 8 to $8\frac{3}{4}$ hours after the onset of anesthesia, corresponding in time of anesthesia with the samples taken before, after 2 hours and after 6 hours of effective hyperthermia. The results obtained on the controls are given in table 2. Curves plotted from the data obtained in one of them are given in figures 2, C and 3. It will be seen from these results that, although the anesthesia *per se* produced a variable lowering of the alkaline reserve, indicated by the lowering of the

TABLE 2

Control experiments—effect of anesthesia without hyperthermia on CO_2 absorption and buffer values of the blood

EXPERIMENT NUMBER	HOURS OF ANESTHESIA	(A) CO_2 CAPACITY IN VOLUMES PER CENT AT pCO_2		(B) $\Delta[CO_2]_{45-15}$		(C) CHANGE IN THE $\Delta[CO_2]_{45-15}$		(D) pH AT pCO_2		(E) $-\Delta pH_{45-15}$	(F) CHANGE IN THE ΔpH_{45-15}	(G) $\frac{\Delta[CO_2]}{-\Delta pH}$	(H) ACTUAL pH AT ALVEOLAR pCO_2
		15 mm.	45 mm.	Volumes per cent	Millimols	Volumes per cent	Millimols	15 mm.	45 mm.				
12	Immediately after	28	51	23	10.35			—	—	—	—	—	—
	2½ hours	27	50	23	10.35	0	0.0	—	—	—	—	—	—
	6 hours	29	51	22	9.9	−1	−0.4	—	—	—	—	—	—
13	Immediately after	36	53	17	7.6			—	—	—	—	—	—
	2 hours	32	51	16	7.2	−1	−0.4	—	—	—	—	—	—
	6 hours	29	46	17	7.6	0	0.0	—	—	—	—	—	—
16	½ hour	37	56	19	8.5			7.54	7.21	0.33		25.8	7.29
	2½ hours	29	39	10	4.5	−9	−4.0						7.29
	4 hours	31	49	18	8.1	−1	−0.4	7.54	7.21	0.33	0.00	24.6	7.27
	8½ hours	31	49	18	8.1	−1	−0.4	7.54	7.20	0.34	+0.01	23.8	7.31
17	½ hour	37	49	12	5.4			7.55	7.20	0.35		15.6	7.32
	2½ hours	34	46	12	5.4	0	0.0	7.54	7.20	0.34	−0.01	15.9	7.30
	4½ hours	33	45	12	5.4	0	0.0	7.53	7.28	0.35	0.00	15.6	7.30
	8½ hours	32	43	11	4.9	−1	−0.5	7.53	7.19	0.34	−0.01	14.4	7.31
Maximum errors....		±0.5		±1.0	±0.45	±1.0	±0.45	±0.01		±0.02	±0.02	±2.0	±0.01

CO_2 absorption curve, this decrease in the CO_2 combining power is practically equivalent at lower and at higher pCO_2. Consequently the slope of neither the CO_2 absorption curve nor of the $\frac{\Delta pH}{\Delta pCO_2}$ curve changes, and the buffering value of the blood is not increased. This is easily seen by comparing the sets of values in column G, table 2, and is clearly demonstrated by the parallel nature of the logarithmic curves plotted in figure 2, C. We are therefore justified in attributing the changes observed to the prolonged increase of body temperature.

As a preliminary study of the possible causes for this increased buffer value of the blood, we determined the total solids and the hemoglobin concentration of the blood. The latter was calculated from the oxygen capacity. Of these two factors, the latter is known to be the most essential. Van Slyke, Hastings and Neal (1922) have shown that about 75 per cent of the buffer value of the blood can be assigned to the buffering by hemoglobin. Furthermore, Peters, Bulger and Eisenmann (1924) found the buffer

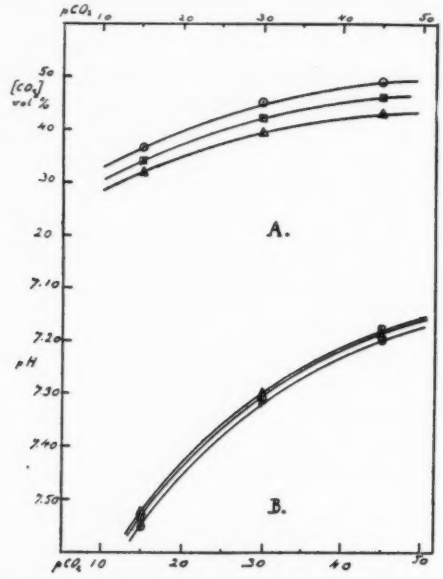

Fig. 3. Curves plotted from data obtained in a control experiment (expt. 17). A: The CO_2 absorption curves or $\frac{\Delta[CO_2]}{\Delta pCO_2}$ curves. B: The $\frac{\Delta pH}{\Delta pCO_2}$ curves. ⊙—⊙: curves of blood after $\frac{3}{4}$ hour anesthesia. ⊡—⊡: curves of blood after $4\frac{3}{4}$ hours' anesthesia. △—△: curves of blood after $8\frac{3}{4}$ hours' anesthesia.

value of the blood to be practically a straight line function of its hemoglobin content and to follow the hemoglobin concentrations very closely when the concentration of the blood changes. For practical purposes, the effect of the changing concentration of the other buffers of the blo od is negligible.

Our determinations on the concentration of the blood, and on its oxygen capacity and hemoglobin content are given in table 3. These data show a definite increase in the concentration of the blood during hyperthermia (column A). The amount of total solids in 100 cc. of blood increases by 4 to 20 per cent of its original value (column C). Other investigators have

also observed an increase in similar cases. Bazett and Haldane (1922) describe an increase in the hemoglobin content of the blood amounting to from 10 to 16 per cent after exposure of men to hot baths for about 20 minutes, and deduce from this a reduction of the volume of the blood produced by evaporation and profuse perspiration. Flinn and Scott (1923) found an increase in blood concentration in dogs after about two hours' hyperthermia.

But it is important to emphasize that in our experiments the increased concentration of the blood is not accompanied by a corresponding increase in the hemoglobin content. Even in a more concentrated blood the oxygen capacity, and consequently the hemoglobin content calculated from it, is below normal. Data in column D show that the oxygen capacity is found to be reduced by 2 to 3.7 volumes per cent, corresponding to a reduction of 1.4 to 2.8 grams of hemoglobin per 100 cc. of blood (column G). The *actual* reduction in hemoglobin must be even greater than the difference between the percentages found in the normal and in the hyperthermic blood because the latter is a per cent value of a more concentrated blood. It seems reasonable to consider the increase in total solids as principally due to a reduction in the water volume. It is logical to consider an increase in the water evaporation in the lungs as a result of the higher temperature and excessive lung ventilation.[3] The possibility of an increase in total solids by the addition of salts or proteins to the serum seems rather remote and can therefore be discarded until proven by direct evidence to be an actual fact. The possibility of an increase in the number of red blood cells seems to be contradicted by the simultaneous reduction in the hemoglobin content. We therefore feel reasonably justified in recalculating the amount of hemoglobin in the hyperthermic blood in terms of the original blood concentration. This was done by multiplying the observed oxygen and hemoglobin values by the ratio $\frac{\text{original concentration}}{\text{actual concentration}}$. From this product we obtain the figures given in table 3, column E, for the recalculated oxygen capacity, and in column H, for the recalculated hemoglobin per cent. When this is done, we find a loss of hemoglobin equal to from 1.5 to 3 grams per cent in two hours of hyperthermia, and 2 to 5.8 grams per cent in 6 hours (column I). We can only conclude, therefore, that about 9 to 36 per cent of the original amount of hemoglobin expressed as oxygen capacity has disappeared from the blood (column J).

In order to be quite certain that the above mentioned changes in blood solids and hemoglobin concentration are really due to hyperthermia it is

[3] The respiratory rate is increased from 20 to 30 (normal) to from 250 to 400 respirations per minute during hyperthermia. This is accompanied by a definite hyperventilation as indicated by the marked lowering of the alveolar CO_2 tension which changes from 30 to 40 mm. (normal) to from 10 to 20 mm. during hyperthermia.

necessary to consider the results obtained in the control animal (expt. 24). The data shown in table 3 indicate only a minimal change in concentration, almost within the limits of error, and a very small change in the hemoglobin content which could be explained by a dilution of the blood with plasma following withdrawal of blood in the consecutive sampling. A simple calculation bears this out. The dog which weighed 11 kilos would contain

TABLE 3

Changes produced by hyperthermia and anesthesia on blood concentration, O_2 capacity, and hemoglobin content

EXPERIMENT NUMBER	HOURS OF HYPERTHERMIA	MAXIMUM TEMPERATURE	CONCENTRATION			O_2 CAPACITY			HEMOGLOBIN CONTENT			(J)
			(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)	
			Total solids	Amount of change	Per cent change	O_2 capacity as found	Recalculated in terms of original concentration	Difference from normal	Hemoglobin as found	Recalculated in terms of original concentration	Difference from normal	PER CENT CHANGE, O_2 CAPACITY AND HEMOGLOBIN
19	Before	37.4	19.5			21.5			16.1			
	2 hours	40.2	22.4	2.9	14.9	20.6	17.9	3.6	15.4	13.4	2.7	16.8
	6 hours	40.2	25.0	6.5	29.0	18.2	13.6	7.7	13.6	10.4	5.8	36.0
20	Before	38.2	21.8			18.4			13.7			
	2 hours	40.8	23.8	2.0	9.2	16.8	15.4[a]	3.0	12.5	11.5	2.2	16.3
	6 hours	40.8	25.0	4.8	12.8	15.3	13.6	4.8	11.4	10.1	3.6	26.0
21	Before	38.0	18.7			19.4			14.5			
	2 hours	41.2	20.3	1.6	8.9	18.2	16.8	2.6	13.6	12.5	2.0	13.4
	6 hours	41.2	18.5	0.2	0.0	15.7	15.7	3.6	11.7	11.8	2.7	19.0
22	Before	36.5	18.6			20.6			15.4			
	2 hours	40.8	19.4	0.8	4.5	19.5	18.7	1.95	14.5	13.9	1.5	9.1
	6 hours	40.8	19.4	0.8	4.5	18.8	18.0	2.6	14.0	13.4	2.0	9.8
24 control	1½ hours anesthesia	37.6	18.5			19.0			14.2			
	4½ hours anesthesia	37.6	18.9	0.4	2.2	18.7	18.2	0.7	14.0	13.6	0.6	3.7
	8½ hours anesthesia	38.0	18.6	0.1	0.5	18.2	18.1	0.9	13.7	13.5	0.7	4.7
Maximum errors			±0.3	±0.3	±1.5	±0.1	±0.4	±0.4	±0.1	±0.4	±0.4	±2.1

approximately 1,000 cc. of blood or 9.2 per cent of the body weight (Smith, Arnold and Whipple, 1921). If the entire 50 cc. of blood withdrawn in the first sample were replaced by plasma, the hemoglobin content would be reduced to $14.2 \times \frac{1,000 - 50}{1,000} = 13.5$, or a loss of about 0.7 gram per 100 cc. of blood. Similarly, the total loss following the drawing of another 50 cc.

sample would be about 1.4 grams per cent. These figures are still larger than the reduction actually observed in the control. But this dilution effect cannot be used to explain the more marked reduction observed in the case of the hyperthermic animals. In these cases dogs weighing 11 to 13.7 kilos show after the withdrawal of the first 50 cc. sample a reduction not of 0.7 but 1.5 to 2.7 grams; in other words, twice to four times as much.

These results demonstrate that the blood during hyperthermia does not behave as normal blood, where, as Peters, Bulger and Eisenmann (1924) found, the buffer value is a direct function of the hemoglobin concentration. On the contrary, an increase in the buffer value is accompanied by a reduction in hemoglobin concentration. Therefore, the increase in buffer value of the blood must be explained in a different way.

A consideration of the figures given in column B of table 1 shows that the amount of base liberated by the hyperthermic blood as the pCO_2 increases from 15 to 45 mm. is much greater than that set free by the normal blood over the same range of pCO_2. Actually there appears in the blood between 3.5 to 6.3 millimols (column C, table 1) of a more labile, more loosely bound base, upon which the increased buffer value depends.

The appearance of this more labile base may be explained on the assumption that the hemoglobin which disappears from the blood as oxygen capacity, is transformed into a substance which holds base with less strength than functional hemoglobin, i.e., a substance with acid properties like hemoglobin but with a smaller acid dissociation constant. While this assumption is not fully proven, it is supported by the following fact: In the three experiments (nos. 20, 21 and 22) in which calculations could be made, it was found that the amount of excess base bound by CO_2 between 15 and 45 mm. pCO_2 seems to correspond to the amount of hemoglobin which disappears from the blood. The quotient: Excess of increment in CO_2 between pCO_2 15 to 45 mm., expressed in millimols (table 1, column C), divided by the amount of hemoglobin which is lost from the blood (table 3, column I), is equal to 2.5, 2.0 and 2.3 respectively.

Finally, it should be added that the change in slope of the CO_2 absorption curve and the corresponding increase in buffering power of the blood are not due to a moribund condition of the animal. The animals survived for long periods of time after taking the sample where the maximum change occurs. Thus in experiment 19 the animal did not die until $4\frac{1}{2}$ hours after the time when the sample whose values are given was taken. In experiments 6 and 7, the animal died only when its temperature was further raised above the critical point, and this only happened after $1\frac{1}{4}$ hours of higher body temperature. In experiments 9, 20 and 22 the animal was alive and apparently unaffected $1\frac{1}{2}$, $6\frac{1}{2}$ and $5\frac{1}{2}$ hours respectively after taking the sample whose values are given.

SUMMARY

1. The temperature of anesthetized dogs was raised by physical means and maintained between 40 and 41°C. for periods longer than 8 hours.

2. Hyperthermia when prolonged beyond two hours produces marked changes in the factors determining the acid-base equilibrium of the blood.

3. A definite increase in the slope of the CO_2 absorption curve is observed. This is accompanied by a decrease in the slope of the $\frac{\Delta pH}{\Delta pCO_2}$ curve. The CO_2 absorption curve of hyperthermic blood crosses the normal curve of the same animal.

4. It is not possible to speak of a decrease nor of an increase in the alkaline reserve as a result of hyperthermia. While the CO_2 capacity at low pCO_2 is lower than normal, indicating a fixed acid acidosis, at high pCO_2, the CO_2 capacity is normal or greater than normal. Hence, it is not possible to obtain a complete picture of the condition of the blood by determining the CO_2 combining power at one single CO_2 tension.

5. The difference in the slope of both the CO_2 absorption and the pH curves causes a very marked increase in the buffering value of the blood for CO_2.

6. Although the increase in the slope of the CO_2 curve and in the buffering value of the blood is accompanied by an increase in the concentration of the blood, it is not associated with, nor produced by, an increase in the hemoglobin content. On the contrary, the hemoglobin content measured as oxygen capacity is reduced by approximately 10 to 30 per cent of the original value.

7. These effects cannot be attributed to the effect of prolonged anesthesia. Chloretone anesthesia, as used, produced a drop in the alkaline reserve without change in the slope of either curve; likewise, no alteration was observed in the buffer value, in the blood concentration, or hemoglobin content.

8. The mechanism of the increase in the buffer value of the blood produced by hyperthermia can be tentatively explained by assuming that the hemoglobin, which disappears as oxygen capacity, is transformed into a non-oxygen carrying substance which holds base less strongly than hemoglobin, and can, therefore, release it more readily to increasing amounts of CO_2.

BIBLIOGRAPHY

ADOLPH, E. F. 1924. This Journal, lxvii, 573.

BANUS, M. G. 1926. This Journal, lxxvi, 216.

BAZETT, H. G. AND J. B. S. HALDANE. 1922. Journ. Physiol., lv, IV.

CAJORI, F. A., C. Y. CROUTER AND R. PEMBERTON. 1923. Journ. Biol. Chem., lvii, 217.

CHEER, S. 1928. This Journal, lxxxiv, 587.

FLINN, F. B. AND E. L. SCOTT. 1923. This Journal, lxvi, 191.
HAGGARD, H. W. 1920. Journ. Biol. Chem., xliv, 131.
HENDERSON, L. J., A. V. BOCK, H. FIELD, JR. AND J. L. STODDARD. 1924. Journ. Biol. Chem., lix, 378.
LANDIS, E. M., W. L. LONG, J. W. DUNN, C. JACKSON AND U. MEYER. 1925. This Journal, lxxvi, 35.
PETERS, J. P., JR. Journ. Biol. Chem., lvi, 745.
PETERS, J. P., JR., H. A. BULGER AND A. J. EISENMANN. 1924. Journ. Biol. Chem., lviii, 747.
SMITH, H. P., H. R. ARNOLD AND G. H. WHIPPLE. 1921. This Journal, lvi, 336.
STADIE, W. C., J. H. AUSTIN AND H. W. ROBINSON. 1925. Journ. Biol. Chem., lxvi, 901.
STADIE, W. C. AND K. A. MARTIN. 1924. Journ. Biol. Chem., lx, 191.
VAN SLYKE, D. D., A. B. HASTINGS AND J. M. NEILL. 1922. Journ. Biol. Chem., liv, 507.

CONCERNING THE SO-CALLED INVERSION EFFECT ON BLOOD PRESSURE OF PREPARATIONS FROM THE POSTERIOR LOBE OF THE PITUITARY GLAND

R. L. STEHLE[1]

From the Department of Pharmacology, McGill University, Montreal

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The blood pressure response produced by repeated injections of extracts of the posterior lobe of the pituitary gland is still a disputed point. Abel and his collaborators (1) maintain that after the first injection of posterior lobe preparations, and even of their pituitary tartrate, subsequent injections often produce a fall of blood pressure, whereas Hogben and Schlapp (2) have maintained that no inversion is obtained if the material is first extracted thoroughly with alcohol to remove a depressor substance or substances which usually contaminates such extracts. Geiling (3) has recently stated that "even after a defatted pituitary powder has been extracted 48 hours in a Sohxlet with absolute alcohol, as described by Hogben and Schlapp, it will still produce, when injected into etherized cats, either with intact or cut vagi, and in the same doses as used by Hogben and Schlapp, a lessened response with the second injection and later injections will effect the inversion. Undoubtedly the presence of depressor substances will facilitate the fall in blood pressure of repeated injections of pituitary extracts, but Hogben and Schlapp are in error when they assert that the fall in pressure is due only to the depressor substance and is not an intrinsic property of the infundibular hormone as is maintained by Abel and his associates." Even more recently, Kamm, Aldrich, Grote, Rowe and Bugbee (4) in their separation of the pressor and oxytocic constituents of the posterior lobe obtained products which they state gave no depressor effect on repeated administration.

We have had occasion during the last few years to prepare more or less purified products from the posterior lobe of the pituitary gland in this laboratory and it appeared desirable to record our experience on the point in dispute. The tracings reproduced in this communication were obtained from a preparation obtained as follows: The whole pituitary glands after removal from cattle were chilled or frozen and brought to the labora-

[1] The author is indebted to Mr. Bruger and Mr. Morris for assistance in some of the experiments herein described, and to Chas. E. Frosst & Co., Montreal, for some of the raw material used.

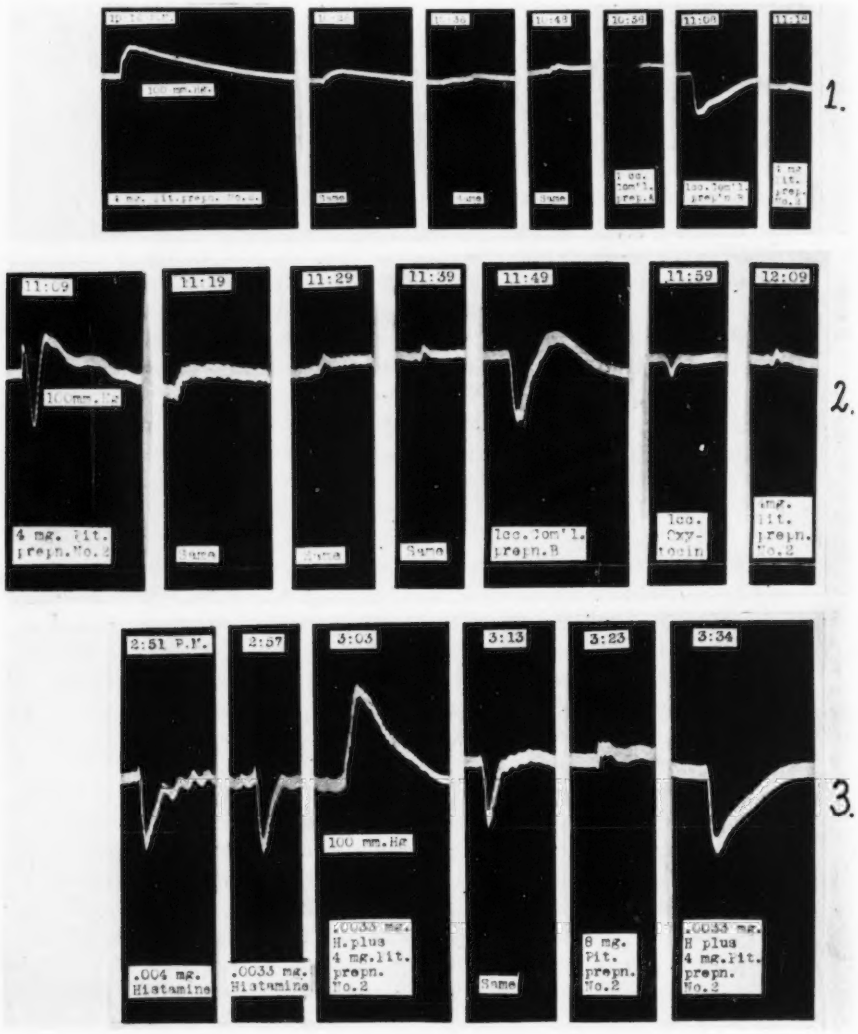
tory. The posterior lobes were then dissected from the cold or frozen gland and dropped into acetone. When convenient (in one case not until two years after the material was placed in acetone) the acetone was removed by filtration and the glands were finely minced in a meat chopper or by trituration in a mortar with ground glass, in the latter case after the addition of water. The finely divided material was then heated with water to the boiling point and the insoluble material separated by centrifuging. The insoluble portion was washed twice by shaking it with water and centrifuging; the washings were combined with the first solution obtained by centrifuging. Colloidal iron was then added to this solution until a perfectly clear filtrate could be obtained. The filtrate was evaporated at about 50° under reduced pressure (usually 25 to 30 mm. Hg) to a small volume and absolute alcohol was added until the flocculent white precipitate which resulted appeared to be completely thrown down. This was separated by centrifuging, the alcoholic solution was discarded and the precipitate washed twice with more absolute alcohol in the centrifuge bottle. Finally the insoluble material was filtered by suction and dried in a vacuum desiccator. After drying it appears to retain its activity well. Preparations which we have obtained by other methods have given results similar to those here recorded. In addition to injections of our own products injections of commercial preparations were also made from time to time.

The following tracings (figs. 1 and 2) are representative of many experiments. Etherized cats were used; blood pressure was recorded from the carotid and all injections were made through a needle inserted into one of the femoral veins. The interval between injections was usually ten minutes. Each solution injected was followed with 2 cc. of saline. Controls were, of course, always carried out; usually there was no saline effect.

In no instance do the experiments indicate that the preparations made as just described produce on second or subsequent injection a fall of blood pressure instead of a rise, i.e., an inversion effect. The only result of the second and subsequent injections, made usually at ten minute intervals, is a rise in pressure, generally smaller than the first, or no pronounced effect of any kind. Whether or not a rise occurs appears to depend upon the size of the first dose. A large dose (8 mgm.) renders the vascular system wholly immune to any rise of blood pressure subsequently while with a smaller initial dose (1 mgm.) considerable pressor effect could again be elicited.

Tracing 2, which is not representative of the results as a whole, shows two blood pressure maxima on first injection and the fall between the rises is to a point below the pressure just prior to the injection. This is not to be regarded as a reversal of action since it occurs with the *first* injection and does not appear with later injections. What caused this effect is not clear but we hope to make the phenomenon the subject of further study. Since

these experiments were performed we have observed this effect frequently in the dog.



Figs. 1–3

The commercial preparations tested (10 International units per cubic centimeter) had in every case some depressor action. Two were American

in origin, one English and one Canadian. Preparation B had an especially strong depressor action as may be seen from figure 1. The others produced effects similar to that shown by preparation A in the same tracing.

The vasopressin of Parke, Davis & Co., which represents the pressor constituents of the posterior lobe also has a *slight* depressor action on second and subsequent injections. Oxytocin (the oxytocic constituent prepared by the same Company) also has a slight depressor action (fig. 2).

An effort was made in another way to shed light on the possibility of contamination being the explanation of the so-called reverse effect. Mixtures of our pituitary preparation No. 2 with histamine were made and the blood pressure effects of repeated injections observed. A representative experiment is shown in figure 3. It may be seen that 0.0033 mgm. of histamine (the bihydrocholoride is meant) alone produced a well marked fall of blood pressure. The mixture of this quantity with 4 mgm. of the pituitary preparation produced a pure rise of pressure but *exactly the same solution* on injection ten minutes later produced a pure fall of pressure. The presence of the histamine was therefore completely covered in the first injection of the mixture. Evidently the blood vessels were rendered immune to further pressor action by this injection but the histamine action came out well with later injections. Eight milligrams of the posterior lobe preparation alone produced only a slight rise of pressure. Finally the effect of the mixture was again obtained, the fall in this case being more pronounced than the first time.

Occasionally the depressor action comes out to a slight extent with the first injection of the histamine-pituitary mixture; that is, a slight fall of blood pressure precedes the rise. Whether or not this will occur probably depends upon the relative concentrations of the histamine and pituitary preparation and the vascular condition of the particular animal.

Our results therefore confirm those of Hogben and Schlapp. That is, they show that posterior lobe preparations can be obtained without great difficulty which have a pressor effect only. We are of the opinion that the inversion effect described by Abel and his collaborators is due to contamination of their products with a depressor substance. The only explanation we can see for Geiling's failure to eliminate the depressor action is that when one attempts to extract the depressor substance from the posterior lobe powder with absolute alcohol the penetration of the solvent may sometimes be too slow or slight to accomplish its complete extraction. When, on the other hand, one precipitates the pressor constituent from a previously purified solution (as described herein) the depressor substance appears to remain in solution and does not contaminate the precipitate.

It does seem rather strange that a depressor substance should contaminate Abel's highly active material (highly active so far as uterine action is concerned) but that it does seems to be the inevitable conclusion to be

drawn from the present experiments. In the present experiments, out of 29 injections of our preparations made subsequent to the first, not a single fall of blood pressure was observed. That we have not injected enough material to bring out the inversion effect does not appear to be a valid criticism. A preparation which will produce such a rise in pressure as that shown in figure 3, e.g., should produce a reverse effect, if there were such an effect. That it does not is quite evident from the tracing.

CONCLUSIONS

Preparations of the posterior lobe of the pituitary gland made as described show no reversal of action upon the blood pressure upon repeated injections at ten minute intervals.

BIBLIOGRAPHY

(1) Abel and Rouiller. Journ. Pharm. Exper. Therap., 1922–23, xx, 65; 1924, xxii, 289.
(2) Hogben and Schlapp. Quart. Journ. Exper. Physiol., 1924, xiv, 229.
(3) Geiling. Physiol. Reviews, 1926, vi, 62.
(4) Kamm, Aldrich, Grote, Rowe and Bugbee. Journ. Amer. Chem. Soc., 1928, l, 573.

ON THE TOXICITY OF PURIFIED BILE PREPARATIONS

EUGENE U. STILL

From the Hull Physiological Laboratory of the University of Chicago

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There has not been a unanimity of opinions concerning the toxic factors found in bile, especially concerning the factors which are responsible for the symptoms observed in obstructive jaundice and in bile peritonitis. Horrall and Carlson (1928) have recently published articles which include bibliographies on this subject, therefore the literature will be dealt with only as it is particularly pertinent to this paper.

Since other workers have not, in the main, worked with bile constituents of known high purity, we have prepared and studied several of them, hoping to determine their respective toxicities. We have prepared a great many bile constituents, but are reporting in this paper only on those prepared in very high states of purity.

PREPARATION OF BILE ACIDS. *Glycocholic acid.* Crude sodium glycocholate (70 per cent) was obtained from Armour and Company. A solution of this salt was precipitated with 10 per cent ferric chloride, subsequently filtered and the precipitate washed, decomposed with sodium carbonate and impure glycocholic precipitated with ether and hydrochloric acid, according to Hammarsten (Handbuch der Biologischen Arbeitsmethoden, VI—Abt. p. 211). The crystals were filtered on a Büchner funnel and washed once with cold water. Then dissolved in absolute alcohol and decolorized with norit. Glycocholic acid was precipitated by adding a large excess of ether and allowing to stand in the refrigerator. The sodium salt was prepared from this product and the above process again followed through. The preparation thus obtained was recrystallized two times from alcohol by adding 5 volumes of water. The product thus obtained was in fine needles and entirely white. Figure 1 A shows a microphotograph of the product and table 1 shows the analysis of two such preparations.

The unconjugated acids. Whole beef bile containing 5 per cent solid NaOH was boiled under a reflux condenser for 24 hours. The mixture was diluted with four volumes of water and an excess of 10 per cent HCl added. After standing for some time a crust of precipitated acids covered the bottom of the dish. The crust was dried on the steam bath and powdered. From this the ammonium salts were prepared (to make 2

per cent solution), treated with norit, and filtered. The free acids were precipitated by adding an excess of dilute HCl. The crude acids were taken up in boiling acetone and after some concentration placed in the ice box. The crystalline mass resulting was filtered on a Büchner funnel and washed once with ice cold acetone. Most of the colored matter remains in the acetone.

The crude acids thus prepared were separated into the more pure forms by means of magnesium and barium precipitation according to the scheme outlined by Schryver (1912). The individual acids were then further

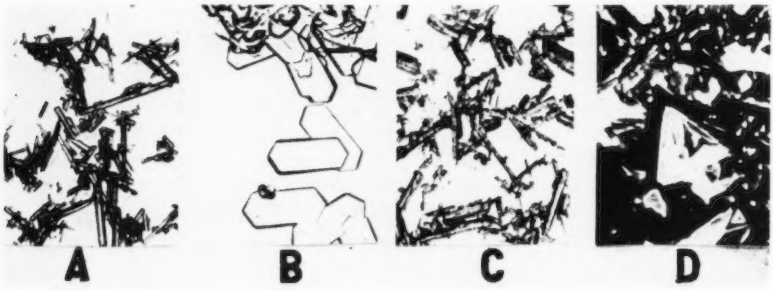

Fig. 1. A—Glycocholic acid—×150; B—Choleic acid—×150; C—desoxycholic acid—×150; D—cholic acid—×150.

TABLE 1

The analyses of glycocholic acid

	TOTAL N	TOTAL P	MELTING POINT (UNCORRECTED)
	per cent	*per cent*	°C.
Theory for $C_{26}H_{43}NO_6$	3.01	0	125–127
Preparation I	3.02	0.010	125–128
Preparation II	2.96	0.023	127–130

purified by various procedures used by H. Wieland and his students (Zeitschr. f. Biol. Chem., 1912, to date). The details of the procedures will be given in a forthcoming paper.

The analytical figures for these acids are shown in table 2. The carbon and hydrogen were determined by combustion; the oxygen by difference; the phosphorus by the method of Fiske and Suboroff. The cholic acid and desoxycholic acid were nitrogen free while I of choleic acid contained a perceptible trace and the other contained about 1 mgm. per cent however, this was only qualitatively measured by means of Nessler's reagent.

Phosphorus was probably present as H_3PO_4; it was the only impurity

present in measurable quantity and only one preparation (choleic acid II) contains a significant amount. This is emphasized by the low melting point of that particular preparation.

Figure 1, B, C and D, shows respectively the microphotographs of our choleic, desoxycholic, and cholic acids.

Preparation of bilirubin. Bilirubin was prepared by a modification of the method of Orndorff and Teeple (1905) from beef gall stones. The fraction less soluble in chloroform was separated and used in these experiments. After this fraction was separated it was dissolved in cold sodium carbonate and a large excess of calcium chloride added. After permitting

TABLE 2

The analyses of the unconjugated acids

	C	H	O	P	MELTING POINT (UNCORRECTED)
	per cent	*per cent*	*per cent*	*per cent*	*°C.*
Cholic acid:					
Theory for $C_{24}H_{40}O_5$	70.53	9.87	19.60	0	198†
Preparation I	70.32	9.74	19.93	0.01	195
Preparation II	69.85	9.78	20.36	0.01	197
Choleic acid:					
Theory for $C_{24}H_{40}O_5$	70.53	9.87	19.60	0	185–187†
Preparation I	70.54	9.68	19.78	0.003*	185
Preparation II	70.02	9.99	19.96	0.03	179
Desoxycholic acid:					
Theory for $C_{24}H_{40}O_4$	73.40	10.27	16.32	0	140‡
Preparation I	73.21	10.45	16.34	0.00	139
Preparation II	72.68	10.04	17.27	0.01	138.5

* Did not give enough color for satisfactory comparison.

† Anhydrous.

‡ From acetone.

the precipitate to settle in the refrigerator the supernatant liquid was decanted. The precipitate was then carried through the complete process of Orndorff and Teeple. This procedure gave a product which was entirely crystalline and contained 9.80 per cent nitrogen. The former authors found their product to contain 9.81 per cent nitrogen. Figure 2 shows a microphotograph of our product.

PHYSIOLOGICAL STUDIES. *On the toxicity of bilirubin.* One gram of bilirubin was suspended in 50 cc. of water and 10 per cent sodium carbonate added (in a closed bottle to prevent oxidation) until solution was complete. This solution was injected intravenously into an unanesthe-

tized dog weighing 5.5 kilograms in about 10 minutes. During the injection and for a few minutes following there was a noticeable respiratory effect due to the introduced alkali. This was quite temporary. The animal was observed for a week without any noted changes due to the injection of bilirubin.

Three guinea pigs (250 grams each) were etherized and a small incision made through the abdominal wall. The intestines were pulled to one side and 0.5, 0.75, 1.0 gram of bilirubin (finely powdered) poured into their respective abdominal cavities. The abdominal wall was closed and 10 cc. of sterile 0.9 per cent NaCl solution injected by needle into the abdomen. All recovered uneventfully. One animal was opened at 48 hours and a great deal of the bilirubin was still present in the cavity. The second was opened at the end of 72 hours and most of the bilirubin had dis-

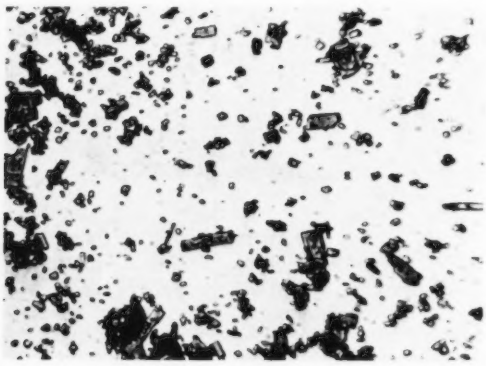

Fig. 2. Bilirubin—×300. Recrystallized from chloroform

appeared. There were no adhesions except at the point of incision. The third animal remained quite normal as long as the observation was continued. There was no sign of the bilirubin in this animal when it was finally opened on the 10th day after the operation.

Due to the insolubility of bilirubin, a direct method of studying the toxicity is not available. However, since Horrall found large amounts of bilirubin did not affect a heart-lung preparation, and since these experiments do not indicate that bilirubin is a toxic agent, else the bilirubin in the guinea pigs would have called forth an ascites, we are of the opinion that pure bilirubin is non-toxic or at least very feebly toxic.

On the toxicity of bile acids. To determine the comparative effects of intravenous injection of molecular equivalents of the various bile acids, the following experiments were carried out: M/4 solutions of the sodium

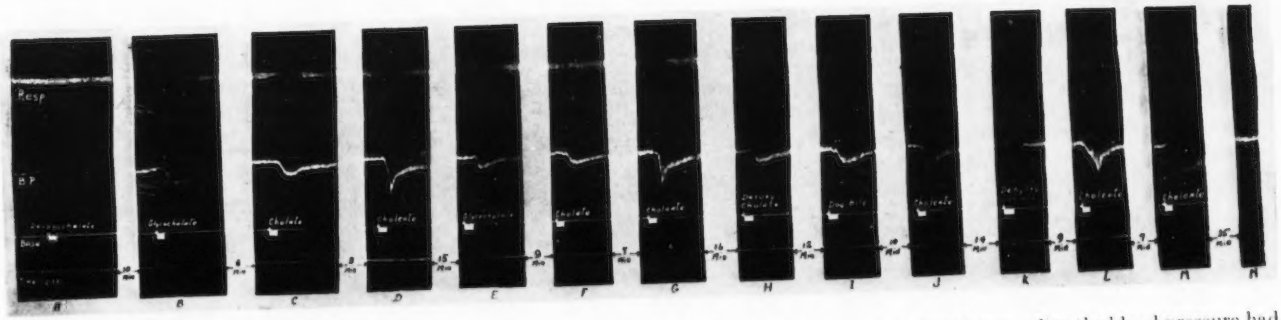

Fig. 3. One cubic centimeter of M/4 sodium salts was injected (in about 30 seconds) intravenously in each case, after the blood pressure had recovered from the previous injection.

salts of glycocholic, cholic, desoxycholic, choleic and dehydrocholic acids were prepared.[1] One cubic centimeter of these salt preparations was injected intravenously in dogs under light barbital anesthesia. Blood pressure and respiration tracings were made. The order in which the injections were made was rotated so that each preparation was injected in a comparable manner to the others. Five dogs were used in these experiments. Figure 3 shows a tracing from one such experiment. Table 3 shows the changes in blood pressure as averaged from all experiments.

An examination of table 3 shows that the order of toxicity is: choleic acid highest, desoxycholic acid next with glycocholic, cholic, and dehydrocholic acids of about the same order of toxicity. Bile is not as toxic as an equivalent quantity of pure bile acids. This, no doubt, is due to protective substances (cholesterol) in the bile.

TABLE 3

Showing the fall in blood pressure caused by the intravenous injection of 1 cc. of M/4 solutions of bile salts (5 dogs)

SALT	NUMBER OF TIMES INJECTED	GREATEST FALL IN BLOOD PRESSURE	SMALLEST FALL IN BLOOD PRESSURE	AVERAGE FALL IN BLOOD PRESSURE
		mm. Hg	*mm. Hg*	*mm. Hg*
Cholate	10	18	15	17
Glycocholate	10	22	12	17
Dehydrocholate	5	19	16	18
Desoxycholate	10	26	17	22
Choleate	20	38	24	30
Bile*	5	16	12	14

* Bile containing an equivalent of 1 cc. of M/4 bile acids.

On injecting these acids in the quantity of 0.1 Mm. per kilogram in dogs, the effect upon respiration is not greatly dependent upon the rate of injection. However, if one injects rapidly (1 minute) the vasomotor effect is very marked, while a slower injection (10 minutes) will hardly cause any immediate effect.

Figure 4 shows the tracings from three dogs in which larger amounts were injected intravenously. Again the greater toxicity of desoxycholate as compared with the cholate is shown. Four-tenths millimol per kgm. of desoxycholate was fatal in 14 minutes. Figure 5 shows the effect of injecting 0.45 mm. per kgm. slowly. A stimulation of respiration and a slight rise of blood pressure is to be noted. Since this animal had received more than a lethal dose of desoxycholate, the experiment shows that the vasomotor effect observed after rapid injections is due either to the momentary high concentration of bile salt, or that during the slow injection the

[1] The very pure dehydrocholic acid was furnished me by Dr. C. H. Green of the Mayo Clinic, and to whom I am greatly indebted.

substance is rapidly removed from the circulation, or both. This view is confirmed by the findings of Green and Snell (1928) who showed that injected bile salts were very rapidly removed from the circulation.

Table 4 shows a compilation on data on frogs used to determine the M. L. D. of the bile salts. The salts were injected into the anterior lymph sac. Frogs weighing between 40 and 50 grams were used (Rana pipiens). This table shows the figures from only a small series but certainly serves

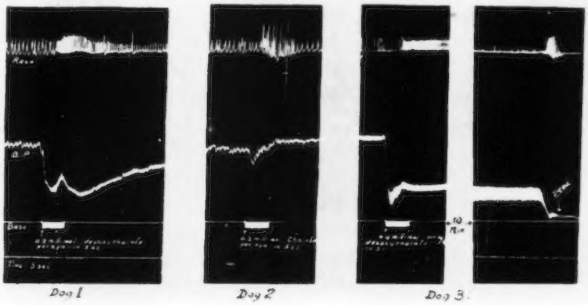

Fig. 4. Effects of injecting large amounts of sodium salts of bile acid intravenously

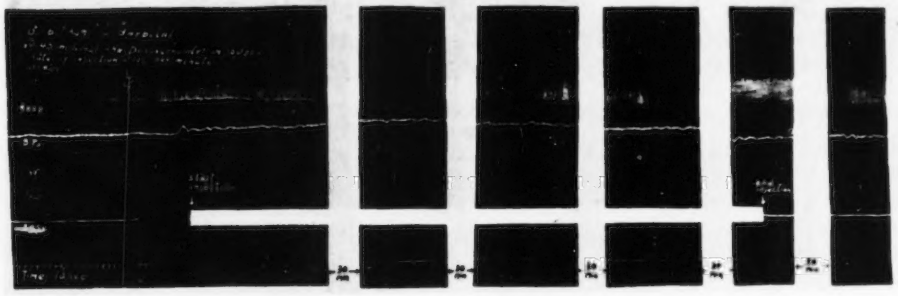

Fig. 5. Effects of slowly injecting intravenously approximately 1½ lethal doses of sodium desoxycholate.

the purpose of showing comparative values for the different bile preparations. This is also in accord with a shorter series of determination by intraperitoneal injections in white rats.

Discussion. In these experiments we have attempted to determine the relative toxicity of bile acids and bilirubin, which we could prepare of a very high purity. This work confirms in every way the work of Horrall and Carlson, that the acids are the most toxic elements in bile and that bilirubin is relatively, at least, non-toxic. Our various tracings indicate

that small amounts of bile acids, slowly injected intravenously, act as stimulants to respiration and cause a slight increase in blood pressure, while larger amounts cause an increased rate of respiration and a marked fall in blood pressure. The vascular effect is not materially affected by the administration of atropin nor by double vagotomy. During a period of very low blood pressure (30 to 40 mm. Hg), the animal responds typically to adrenalin. For these reasons we believe these bile acids to produce their effects not through some special tissue but rather due to their being general protoplasmic poisons.

Since there is no strictly reliable evidence to show that the Van den Bergh reaction on blood of jaundiced human patients bears a constant relationship to the concentration of the more toxic bile acids in the blood, one may doubt the value of that reaction in determining the degree of progress in cases of jaundice.

TABLE 4

Showing the toxicity of bile salts injected into the anterior lymph sac of frogs

The time was six hours and the dosage given in millimols per kilogram of weight.

SALT	0.8 mM.		0.4 mM.		0.3 mM.		0.2 mM.		0.1 mM.		0.05 mM.	
	Number used	Per cent died	Number used	Per cent died	Number used	Per cent died	Number used	Per cent died	Number used	Per cent died	Number used	Per cent died
Cholate	4	100	6	50	6	16	6	0	10	0		
Glycocholate			6	66	6	50	6	16	10	0	5	0
Dehydrocholate			3	33	3	33	3	0	1	0	1	0
Desoxycholate	2	100	2	100	5	100	10	100	10	90	10	10
Choleate	2	100	10	100	5	100	10	100	10	80	10	10

The author wishes to express his appreciation to Dr. A. J. Carlson for reading and criticizing this paper.

CONCLUSIONS

1. Bilirubin is a non-toxic substance.
2. The bile acids are the most toxic substances found in bile.
3. As determined by intravenous injection in the dog, intraperitoneal injection in the rat, or intra-lymphatic injection in the frog, the order of toxicity of the naturally occurring bile acids is: 1, choleic; 2 desoxycholic; 3 glycocholic; 4 cholic.
4. The toxicity of bile lies in the cholate radical.

BIBLIOGRAPHY

GREEN AND SNELL. 1928. Journ. Biol. Chem., lxxviii, 691.

HORRALL, O. H. AND A. J. CARLSON. 1928. This Journal, lxxxv, 591; Arch. Int. Med., in press.

ORNDORFF AND TEEPLE. 1905. Amer. Chem. Journ., xxxii, 215.

SCHRYVER, S. B. 1912. Journ. Physiol., xliv, 265.

THE EFFECT OF SPLENIC CONTRACTION INDUCED BY FARADIC STIMULATION ON THE LEUCOCYTE LEVEL OF THE SPLENIC VEIN

VALY MENKIN[1]

From the Henry Phipps Institute, University of Pennsylvania, Philadelphia

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The possible rôle of the spleen as a factor in modifying the leucocyte level in the circulation has received some attention in the past few years. It has recently been reported that electrical stimulation of the spleen produces in the dog under chloral narcosis a leucopenia in the splenic vein as well as in the general circulation (Viale and Bruno, 1927; Viale, 1927). According to these investigators this leucopenia is accompanied by no change in the differential formula. Binet and Verne (1927), on the other hand, have reported a leucocytosis of short duration following asphyxia. This leucocytosis did not take place after splenectomy. It was consequently interpreted as due to a discharge along with the red blood corpuscles from the splenic pulp during increased contractions of the organ when the animal was exposed to asphyxia. Recently it has been shown that intravenous injection of sodium nucleinate induces a leucopenia, which is followed later by a leucocytosis (Doan, Zerfas, Warren, and Ames, 1928). According to these investigators this leucopenia is caused by the accumulation of neutrophilic leucocytes in the parenchyma of the spleen. In splenectomized rabbits no leucopenia developed, but instead a leucocytosis due to a direct action on the bone marrow. It has been shown (Menkin, 1928) that, whereas in normal cats excitement produced a relative mononucleosis, the increase averaging 13 per cent, the same stimulus in sympathectomized or splenectomized animals not only failed to produce a rise, but subsequently there was even a fall in the relative mononuclear count. No adequate explanation could be found for this subsequent fall in the percentage of mononuclears. The relative mononucleosis after excitement in normal animals was interpreted as probably due to a discharge from the spleen of mononuclears during contraction of the organ. The mechanism involved has been thought to be precisely similar to the one involved in the discharge of red blood corpuscles by the contracting spleen as described by Barcroft (1923, 1927) and more recently by Izquierdo and Cannon (1928).

The present work was undertaken with two main purposes in view; first,

[1] Fellow in Medicine, National Research Council.

to determine whether a relative mononucleosis takes place in the circulation when the spleen is made to contract by direct stimulation; in the second place, to find out by comparison with absolute counts whether this relative increase in mononuclears is due simply to a discharge from the spleen or whether some other mechanism in splenic physiology is involved.

METHOD. The animals used were normal rabbits. A laparotomy was performed under ether anesthesia and the spleen and splenic vein were quickly exposed with as little handling of the organ as possible. A blood sample for a smear and absolute count was immediately removed from the splenic vein by puncturing the latter either wih a capillary pipette or with a fine hypodermic needle (gauge no. 24). In two or three experiments the blood samples were obtained from one of the numerous small veins connecting the spleen directly with the main splenic vein. Immediately after the removal of the basal blood sample the spleen was stimulated directly with a faradic current[2] for varying periods of time ranging from 10 seconds to two minutes depending on the particular experiment. It has been pointed out by Barcroft (1928) that electrical stimulation of the spleen causes it to contract. Since this is a comparatively simple way of maintaining a constant stimulus during an experiment, this method was chosen for eliciting splenic contractions. Under electrical stimulation the spleen of the rabbit could usually be shown to shrink somewhat in size and to become firmer. The margins of the organ often became paler and the purplish color of the parenchyma was usually replaced by a bright red color. Sometimes, however, the central area of the spleen appeared even darker and evidently more congested immediately after stimulation. The latter was especially true if the period of stimulation had been prolonged. At the end of stimulation another sample of blood for a differential and absolute count was immediately collected from the splenic vein.

If previous to the experiment or during anesthesia the animal has been very much excited, the change in the leucocyte level before and after stimulation of the spleen may be much reduced. It is also important to obtain the basal level immediately after exposure of the splenic vein before any marked shrinkage of the spleen has taken place. The second blood sample should be collected immediately after the end of stimulation, inasmuch as a few minutes later the contracted spleen may regain its normal rhythm producing a different effect on the leucocyte level.

Twenty-two animals were used. All, except one, showed consistent changes in the absolute leucocyte level of the splenic vein immediately after stimulation. Three animals did not show any changes in the differential formula after stimulation. Two of these three animals, however,

[2] The secondary coil was placed from 2.5 to 4 cm. from the primary. The primary current used had a potential difference of 3 volts.

were found to be abnormal as shown by post-mortem examination. The smears were made by the ordinary Wright method. Usually 200 cells per smear were counted from which the percentages of polymorphonuclears, lymphocytes and large mononuclears were obtained. The lymphocytes and large mononuclears for the purpose of this experiment were grouped together under the term "mononuclears."

Effect of splenic stimulation for 1 to 2 minutes on the relative mononuclear count of the splenic vein. Faradic stimulation for one to two minutes of the spleen of normal rabbits produces a definite increase in the percentage of mononuclears in the splenic vein, the average increase being 13.6 per cent.

RABBIT NUMBER	PERCENTAGE OF MONONUCLEARS BEFORE STIMULATION	PERCENTAGE OF MONONUCLEARS IMMEDIATELY AFTER STIMULATION
1	47.5	66.5
2	76.0	96.0
3	73.5	97.0
4	29.0	41.5
5	69.0	77.0
6	56.0	66.0
7	37.0	51.0
8	64.5	88.0
9	42.0	62.0
10	39.5	53.0
11	80.0	84.0
12	68.0	77.5
13	52.0	57.0
14	38.0	45.0
Average.....	55.1	68.7

In 14 animals the values for relative mononuclear increase range from 4 to 23.5 per cent. These variations, however, are not surprising when one recalls individual differences in the size and therefore the content of the spleen in rabbits. In this connection it may be noted that in a previous study (Menkin, 1928) emotional excitement for 10 to 15 minutes produced in cats a relative mononucleosis in the peripheral vessels averaging 13 per cent. It is probable that the occurrence of the same average in the rabbit and in the cat by the use of an entirely different technique is purely a coincidence; though it is somewhat suggestive that splenic contractions can cause in the general circulation a change in the differential formula of a relatively constant magnitude.

Effect of splenic stimulation for about 1 minute on the absolute leucocyte count. In order to determine what actually caused this relative mononu-

cleosis in the splenic vein after faradic stimulation of the spleen, a series of experiments was performed in which the absolute leucocyte count was determined.

RABBIT NUMBER	LEUCOCYTES PER CUBIC MILLIMETER BEFORE STIMULATION	LEUCOCYTES PER CUBIC MILLIMETER IMMEDIATELY AFTER ABOUT 1 MINUTE OF STIMULATION
3	6,600	3,300
15	6,850	5,500
4	14,700	5,600
5	14,150	7,000
16	14,850	12,950
7	8,000	5,700
17	15,400	4,100
13	16,100	12,000
14	17,800	13,800
Average.....	12,716.5	7,772.2

It is seen that stimulation for about 1 minute is followed by a leucopenia in the splenic vein. The average fall in the absolute level per cu.mm. is about 4,944 which is a decrease of 39 per cent of the original level.

TABLE 1

Analysis of the changes in the leucocyte level of the splenic vein as a result of about 1 minute of splenic stimulation

RABBIT	ABSOLUTE NUMBER OF LEUCOCYTES PER CUBIC MILLIMETER BEFORE STIMULATION	ABSOLUTE NUMBER OF LEUCOCYTES PER CUBIC MILLIMETER AFTER STIMULATION	ABSOLUTE NUMBER OF POLYMORPHONUCLEARS PER CUBIC MILLIMETER BEFORE STIMULATION	PERCENTAGE OF POLYMORPHONUCLEARS BEFORE STIMULATION	ABSOLUTE NUMBER OF POLYMORPHONUCLEARS PER CUBIC MILLIMETER AFTER STIMULATION	PERCENTAGE OF POLYMORPHONUCLEARS AFTER STIMULATION	ABSOLUTE NUMBER OF MONONUCLEARS PER CUBIC MILLIMETER BEFORE STIMULATION	PERCENTAGE OF MONONUCLEARS BEFORE STIMULATION	ABSOLUTE NUMBER OF MONONUCLEARS PER CUBIC MILLIMETER AFTER STIMULATION	PERCENTAGE OF MONONUCLEARS AFTER STIMULATION
				per cent		*per cent*		*per cent*		*per cent*
3	6,600	3,300	1,749	26.5	99	3.0	4,851	73.5	3,201	97.0
4	14,700	5,600	10,437	71.0	3,276	58.5	4,263	29.0	2,324	41.5
5	14,150	7,000	4,386	31.0	1,610	23.0	9,764	69.0	5,390	77.0
7	8,000	5,700	5,040	63.0	2,793	49.0	2,960	37.0	2,907	51.0
13	16,100	12,000	7,728	48.0	5,160	43.0	8,372	52.0	6,840	57.0
14	17,800	13,800	6,764	38.0	4,554	33.0	11,036	62.0	9,246	67.0
Average..	12,891.6	7,900	6,017.3	46.3	2,915.3	34.9	6,874.3	53.7	4,984.7	65.1

Table 1 shows a further analysis of the mechanism involved after about 1 minute stimulation. It is clear by comparing columns 4, 6, 8 and 10 that

the resulting leucopenia after stimulation involves both a decrease of polymorphonuclears and of mononuclears in the splenic vein. Now, it is also seen by subtracting the figures of column 6 from those in column 4 that the average decrease of polymorphonuclears in the splenic vein after stimulation of the spleen is 3,102. A comparison of columns 10 and 8 shows that the average decrease of mononuclears is only 1,889. In other words, about 62 per cent of the total fall in leucocytes after 1 minute of splenic stimulation is due to polymorphonuclears and only about 38 per cent to mononuclears. It is also noted that before stimulation there were more mononuclears (53.7 per cent) in the splenic vein than polymorphonuclears (46.3 per cent). In spite of this the retention of polymorphonuclears by the stimulated spleen is almost twice that of mononuclears. Evidently the relative mononucleosis after splenic stimulation for about 1 minute seems to be due to a greater retention of polymorphonuclears than mononuclears by the contracted spleen.

Effect of splenic stimulation for short duration (10 to 15 seconds). It is well known from the work of Barcroft (1923, 1926, 1926a, 1927) that the contracting spleen discharges at least a part of its pulp content of red blood corpuscles. It is also known that the pulp of the spleen contains a high concentration of monocytes and lymphocytes (Witts and Webb, 1927). In cats the mononuclear level of the splenic pulp runs as high as 65 per cent whereas the level in the general circulation is about 30 per cent (Menkin, 1928). It was therefore thought that when the spleen discharges its pulp content during the phase of contraction the leucocyte count of the splenic vein might temporarily rise owing to an excessive mononuclear discharge from the splenic pulp. For this reason the time of faradic stimulation of the spleen was decreased to about 10 to 15 seconds in order to see whether a short phase of actual leucocytosis could be obtained in the splenic vein.

RABBIT NUMBER	LEUCOCYTES PER CUBIC MILLIMETER BEFORE STIMULATION	LEUCOCYTES PER CUBIC MILLIMETER IMMEDIATELY AFTER 10 TO 15 SECONDS OF STIMULATION
8	17,600	20,000
9	23,400	25,800
10	9,200	11,400
18	11,000	16,400
19	10,400	11,200
12	6,800	12,600
Average	13,066.6	16,233.3

It is seen that stimulation for 10 to 15 seconds produces a rise in the total leucocyte level, the increase averaging 3,166.7 or 24.2 per cent of the basal level. The results are thus directly opposite to those obtained after 1

minute of stimulation with respect to the absolute leucocyte count. With short stimulations a relative mononucleosis takes place in the splenic vein just as it does with the longer period of stimulation.

An analysis of the exact changes in the splenic vein after stimulation of short duration was made and is shown in table 2. It is seen that such stimulation is accompanied by a discharge of mononuclears and a retention of

TABLE 2

Analysis of the changes in the leucocyte level of the splenic vein as a result of stimulation of the spleen for short duration (10 to 12 seconds)

RABBIT	ABSOLUTE NUMBER OF LEUCOCYTES PER CUBIC MILLIMETER BEFORE STIMULATION	ABSOLUTE NUMBER OF LEUCOCYTES PER CUBIC MILLIMETER AFTER STIMULATION	ABSOLUTE NUMBER OF POLYMORPHONUCLEARS PER CUBIC MILLIMETER BEFORE STIMULATION	PERCENTAGE OF POLYMORPHONUCLEARS BEFORE STIMULATION	ABSOLUTE NUMBER OF POLYMORPHONUCLEARS PER CUBIC MILLIMETER AFTER STIMULATION	PERCENTAGE OF POLYMORPHONUCLEARS AFTER STIMULATION	ABSOLUTE NUMBER OF MONONUCLEARS PER CUBIC MILLIMETER BEFORE STIMULATION	PERCENTAGE OF MONONUCLEARS BEFORE STIMULATION	ABSOLUTE NUMBER OF MONONUCLEARS PER CUBIC MILLIMETER AFTER STIMULATION	PERCENTAGE OF MONONUCLEARS AFTER STIMULATION
				per cent		*per cent*		*per cent*		*per cent*
8	17,600	20,000	6,248	35.5	2,400	12.0	11,352	64.5	17,600	88.0
9	23,400	25,800	13,572	58.0	9,804	38.0	9,828	42.0	15,996	62.0
10	9,200	11,400	5,566	60.5	5,358	47.0	3,634	39.5	6,042	53.0
Same animal 2 minutes later*	9,200	9,100	5,566	60.5	4,232	46.5	3,634	39.5	4,868	53.5
19	10,400	11,200	5,876	56.5	5,768	51.5	4,524	43.5	5,432	48.5
Same animal 13 minutes later*	10,400	8,400	5,876	56.5	2,898	34.5	4,524	43.5	5,502	65.5
12	6,800	12,600	2,176	32.0	2,835	22.5	4,624	68.0	9,765	77.5
Average	13,480	16,200	6,687.6	48.5	5,233.0	34.2	6,792.4	51.5	10,967.0	65.8

* Some hemorrhage took place for 2 minutes (rabbit 10) and for 13 minutes (rabbit 19) before these samples were taken (see text). These figures were not included in the calculation of the average.

polymorphonuclears (columns 4, 6, 8, and 10 in table 2). It is interesting to note in rabbits 10 and 19 of table 2 that prolonged hemorrhage causes a leucopenia after the short period of leucocytosis has passed. Barcroft (1927) has shown that hemorrhage increases the contraction of the spleen. The leucopenia after hemorrhage shows a greater retention of polymorphonuclears (table 2, rabbits 10 and 19) than after 10 to 15 seconds of electric stimulation. This seems to show that not only an electric stimulus

but even a totally different stimulus, such as hemorrhage which nevertheless equally causes splenic contraction, will give rise precisely to the same picture. This picture in the splenic vein is primarily one of polymorphonuclear retention and mononuclear discharge (table 2). Only in one experiment, shown in table 2, there is no retention of polymorphonuclears after 10 seconds of stimulation, but it is clear at the same time that in this experiment (rabbit 12) the discharge of mononuclears is about 8 times that of the polymorphonuclears. The average figures in table 2 show that 10 to 15 seconds of faradic stimulation produce a retention of 1,454 polymorphonuclears (cf. columns 4 and 6) and a discharge of 4,174 mononuclears (cf. columns 8 and 10). The result is a slight increase in the absolute leucocyte level of the splenic vein which is due to the difference between the discharge of mononuclears and the retention of polymorphonuclears, i.e., 2,720 white cells.

Discussion. The foregoing results bring out two facts clearly. First, the stimulated spleen when contracted causes a change in the differential formula of the splenic vein. The result of this qualitative change is a relative mononucleosis. Second, the stimulated spleen when contracting changes the absolute level of the leucocytes in the splenic vein. If the stimulus causing splenic contraction is of short duration a leucocytosis follows. If the stimulus is applied for a longer time the spleen seems to shut down and a leucopenia follows. When the stimulus is of short duration the leucocytosis is caused by a discharge of mononuclears from the splenic pulp. Simultaneous to this discharge of mononuclears, the spleen seems to be able to retain polymorphonuclears (table 2). If the stimulus is applied for a longer time a leucopenia follows in the splenic vein; but analysis of this leucopenia reveals a greater retention of polymorphonuclears than mononuclear cells (table 1). The function of the spleen 10 to 15 seconds after the beginning of contraction seems to be primarily one of mononuclear discharge but at the same time there is some retention of polymorphonuclears (table 2). When the splenic contraction is maintained for a longer interval (about 1 minute) a general retention of all the white cells takes place (table 1); but the retention is unequal, the polymorphonuclears being more affected than the mononuclears. The initial leucocytosis resulting after short stimulation of 10 to 15 seconds is, as pointed out above, explainable as due to a contraction and discharge of mononuclears from the splenic pulp which contains these cells in abundance.

The greater retention of polymorphonuclears than mononuclears by a spleen which is maintained in a contracted state for 1 to 2 minutes may be due to a large addition to the splenic pulp of mononuclear cells from the Malpighian follicles during the initial stage of contraction. The basal

ratio of polymorphonuclears to mononuclears is consequently changed with this abundant addition of mononuclears. As retention of leucocytes takes place with an increase in the duration of contraction, the polymorphonuclears will appear to be much more affected since none have been added to the splenic pulp during the initial contraction.

Roy (1881) pointed out the importance of the rhythmic contractions of the smooth muscular fibres of the splenic capsule and trabeculae in maintaining the circulation through the organ. Roy also showed that the spleen when in a powerful state of contraction loses its natural rhythmical contraction and expansion. This would consequently involve a decrease in the intrasplenic circulatory rate. During the prolonged maintenance of contraction which occurs when the spleen is stimulated from 1 to 2 minutes, it is conceivable that many of the cellular elements of the blood may actually stagnate and remain caught in the splenic pulp owing to the decreased circulatory rate within the organ. This would account for the leucopenia in the splenic vein immediately after one minute of stimulation.

In a previous publication (1928) it was shown that the relative mononucleosis of normal cats was not seen in sympathectomized or splenectomized cats. On the contrary these animals after prolonged excitement showed a relative decrease in the percentage of mononuclears. No adequate explanation could be offered at the time. It is believed now that this relative decrease of mononuclears was due to a relative increase in polymorphonuclears most likely from the bone marrow which could not be retained in a splenectomized or in a sympathectomized animal where the spleen was denervated and consequently could not contract.

A clinical condition, in which a relative lymphocytosis and a leucopenia are seen, is hyperthyroidism (Kocher, 1908). It has been shown that patients with exophthalmos, in whom, presumably, the sympathetic division of the autonomic system is abnormally stimulated, show a greater relative lymphocytosis than patients having hyperthyroidism without any abnormal eye signs (Menkin, 1928a). In such cases increased sympathetic activity would presumably increase the amount of splenic contraction and consequently intensify the relative lymphocytosis. The accompanying leucopenia as described originally by Kocher would possibly be due to the general unequal retention of leucocytes by a maintained contracted spleen.

Since the spleen is under sympathetic control, many factors involved in the activation of this system, as shown within the last few years by Cannon and his collaborators, such as emotions, muscular activity, cold, pain, asphyxia, or hemorrhage would presumably cause the spleen to increase its contractions. The spleen would therefore become a potent factor in the modification of the leucocyte level by continuous changes in its contractility depending in turn on sympathetic impulses. The well known daily fluctua-

tions of white blood cells may largely be due to changes in splenic activity. There are probably other factors coming into play to modify the level of white blood cells, such as the activity of the bone marrow and lymph nodes. The spleen per se, however, is undoubtedly an important organ in causing by its contractions quick qualitative and quantitative variations in the leucocyte picture of the circulation.

SUMMARY

1. In normal rabbits, faradic stimulation of the spleen for 1 to 2 minutes causes this organ to contract. This is accompanied by an increase in the percentage of mononuclears of the splenic vein, averaging 13.6 per cent.

2. Stimulation of the spleen for about 1 minute is followed by a leucopenia in the splenic vein, the decrease in the absolute leucocyte count averaging 4,944 cells.

3. The relative mononucleosis in the splenic vein after stimulation of the organ for about 1 minute is due to a greater retention of polymorphonuclears (62.1 per cent) than mononuclears (37.9 per cent) by the spleen when contracting (table 1).

4. Stimulation with the same strength current but for shorter intervals of time (10 to 15 seconds) produces a leucocytosis in the splenic vein, the increase in the absolute count averaging 3,166 cells. This initial leucocytosis is the resultant of a greater discharge of mononuclears from the spleen than retention of polymorphonuclear cells (table 2).

5. The spleen, while contracting, is able to change the differential leucocyte formula first by discharging primarily mononuclear cells from its pulp (table 2), and secondly, in the course of continued contraction by lowering the absolute leucocyte level in the circulation through an unequal retention, involving the polymorphonuclears to a larger extent than the mononuclears (table 1). Similarly the spleen contracting in response to sympathetic stimulation plays an important part in modifying the leucocyte picture of the circulation (1928).

BIBLIOGRAPHY

BARCROFT, J. AND H. BARCROFT. 1923. Journ. Physiol., lviii, 138.

BARCROFT, J. 1926. Ergebn. d. Physiol., xxv, 818.

1926a. The Lancet, ccx, 544.

1927. Le Sang, i, 97.

BARCROFT, J. AND H. W. FLOREY. 1928. Journ. Physiol., lxvi, 231.

BINET, L. AND J. VERNE. 1927. Journ. Physiol. et Path. Gén., xxv, 638.

CANNON, W. B. AND S. W. BRITTON. 1927. This Journal, lxxix, 433.

CANNON, W. B., A. QUERIDO, S. W. BRITTON AND E. M. BRIGHT. 1927. This Journal, lxxix, 466.

DOAN, C. A., L. G. ZERFAS, S. WARREN AND O. AMES. 1928. Journ. Exper. Med., xlvii, 403.

IZQUIERDO, J. J. AND W. B. CANNON. 1928. This Journal, lxxxiv, 545.
KOCHER, T. 1908. Arch. f. Klin. Chir., lxxxvii, 131.
MENKIN, V. 1928. This Journal, lxxxv, 489.
1928a. Arch. Int. Med., xlii, 419.
ROY, C. S. 1881. Journ. Physiol., iii, 203.
VIALE, G. AND A. A. BRUNO. 1927. C. R. Soc. Biol., xcvii, 1238.
VIALE, G. 1927. Arch. d. Fisiol., xxv, 380.
WITTS, L. J. AND R. A. WEBB. 1927. Journ. Path. and Bact., xxx, 687.

I. THE OVARY IN EXPERIMENTAL HYPO- AND HYPERTHYROIDISM

II. THE INFLUENCE OF EXPERIMENTAL HYPERTHYROIDISM ON GESTATION[1]

M. M. KUNDE, A. J. CARLSON AND T. PROUD

From the Hull Physiological Laboratory of the University of Chicago

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Until recently, very little in the way of definite statements concerning the influence of hyperthyroidism on pregnancy, or vice versa, could be found in the literature. Even now the evidence at hand is of an unsatisfactory character and consists mostly of necessarily inadequate clinical observations of a relatively few cases of pregnancy complicated with hyperthyroidism. For recent reports of these cases the reader is referred to articles by R. S. Smith (1928) and Wayne A. Yoakum (1928). Mussey et al. (1926) conclude that in their experience the course of pregnancy and the maternal and fetal mortality were not appreciably affected by the exophthalmic goiter, or the adenomatous goiter with hyperthyroidism; Daly and Strouse (1925) review twenty-five cases and advocate the use of iodine in hyperthyroidism during pregnancy. Basal metabolism determinations were not made on their patients consequently in some of their cases the diagnosis is questionable. Davis (1926) reviews the literature and presents data on a large group of women, some with pregnancy and normal thyroids, others with thyroid hypertrophy with or without symptoms of hyperthyroidism. Howard (1922) states that pregnancy may take place notwithstanding the existence of a moderate form of hyperthyroidism, and in these cases the pregnancy seems to decrease the hyperthyroid symptoms rather than otherwise. This same author quotes Charcot (1885) and Dock (1915) as having observed that sometimes the symptoms of hyperthyroidism appear during pregnancy or the puerperium, while on the other hand many patients improve during pregnancy, and do not afterwards relapse. The above citations refer entirely to spontaneous hyperthyroidism and consequently may have little bearing on the part of this research which deals with hyperthyroidism in pregnancy, in as much as our observations were made on animals which were fed desiccated thyroids until the symptoms of severe hyperthyroidism were evident.

[1] This work has been conducted under a grant from the Douglas Smith Foundation for medical research at the University of Chicago.

Guedernatsch (1915) fed fresh thyroids to rats until emaciation, diarrhea, muscular weakness, and in some instances death occurred; the above signs were accompanied by changes in the coat, i.e., "the hair became yellow, stands on ends, sometimes falls out in patches, and the entire coat looks ragged." In these animals mating usually produced no results; pregnancy was always delayed since fertilization did not occur until several weeks after the administration of the thyroid was discontinued. If pregnancy finally did occur it resulted in either abortion of the young, or the young died soon after birth. In late pregnancy the young showed a diminished tendency to grow, and although not fragile kept in relative size behind offspring of normal rats.

Hoskins (1910) subjected pregnant guinea pigs to thyroid feeding experiments with the intent of influencing the gland weight of their offspring. The variations in susceptibility to the ingested thyroid were demonstrated by the following results: Animal 297 died of hyperthyroidism after eight days of feeding 0.01 gram per day, whereas animal 91 survived for the same period of time on a dose twenty-five times as great. This author incidentally noted that abortions were frequent in the surviving animals.

In our studies on experimental cretinism we observed, as others had reported, that the ovaries of cretin rabbits present a histological picture which differs in many important respects from that of the normal rabbit. Hofmeister (1893–94) observed in rabbits eight months old, and thyroidectomized at four and one-half months after birth that cross sections of their ovaries showed very large follicles closely packed together. He describes premature ripening of the follicles and a condition which Zligler called "follikuläre hypertrophie." It should be remembered that Hofmeister's rabbits were more than half grown when thyroidectomized, even so, the disturbance in ovarian development was marked. Tatum (1915) also noted the large size of the follicle space, and the absence of normal ova in the entire section which he described.

We produced cretin rabbits by removing the thyroids approximately three weeks after birth,[2] and allowing these animals to remain in the cretin condition several months. They were then fed desiccated thyroids in varying amounts (60 mgm. to 140 mgm. per day) over a period of time four to eight weeks in duration, or until the symptoms of severe hyperthyroidism were apparent. Histological sections of the ovaries then revealed large numbers of primary follicles and apparently normal Graafian follicles

[2] In our experience pronounced cretins most frequently resulted if the thyroids were removed before the animals were three weeks old; complete thyroidectomy within two weeks after birth usually results in death from parathyroid tetany even though the lower pair of parathyroids remain intact. This is an interesting phenomenon in as much as the same type of operation performed approximately one week later is not followed by symptoms of parathyroid deficiency.

in all stages of development. (Compare A and B, fig. 1.) The significance of these observations becomes more important when associated with our present knowledge of the development of the primordial ovum.

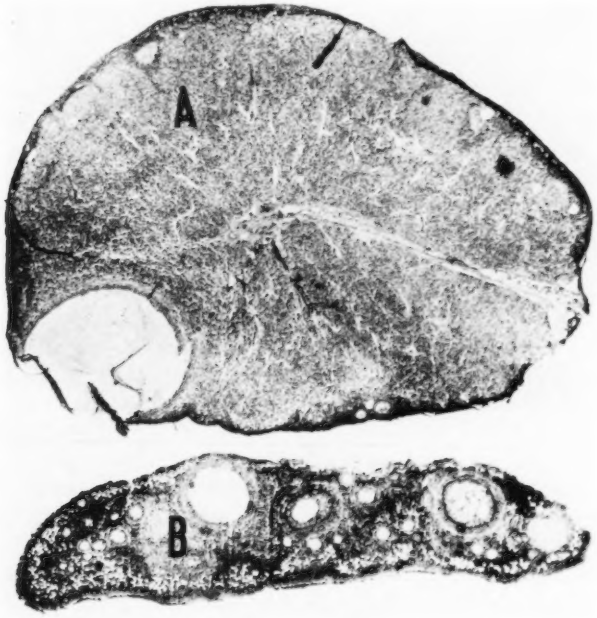

Fig. 1A. Cross section through the ovary of an untreated cretin rabbit, born March 19, 1923. Operated April 4, 1923. Autopsied March 2, 1924; body weight at autopsy 2020 grams.

Fig. 1B. Cross section through the ovary of a cretin rabbit with severe hyperthyroidism induced by the administration of desiccated thyroid. Data taken from protocols: Born February 25, 1924. Operated March 14, 1924. Autopsied July 9, 1924; body weight = 655 grams; very marked cretin, pot belly, apathetic appearance, shaggy coat, hind legs seem very weak, scarcely able to walk. May 31st to June 2nd, cretin received 132.9 mgs. desiccated thyroid daily. Thyroid discontinued June 3rd, because of severe diarrhea. June 15, 1924, much improved in appearance and walks very well. Thyroid treatment again instituted, 66.4 mgs. daily from June 15th to July 8th. Body weight July 3rd = 770 grams, July 8th = 700 grams. Thyroid again discontinued because of severe diarrhea. July 9th, body weight 560 grams. Ovaries removed for histological sections.

The accepted theory relative to the development of the egg cells holds that these cells are present in the ovary in their final number at birth. Minot (1892) states that at all ages small egg cells together with their

follicles present a constant appearance and remain for a long time without change, but from time to time, certain ones develop. In a pronounced cretin, thyroidectomized at three weeks of age, and allowed to reach maturity in a marked hypothyroid condition, only a few follicles are seen near the surface of the ovary, but in the cretin rendered hyperthyroid, great numbers especially of the primary follicles, are easily seen. These essential facts seem contradictory to the theory that all the cells are preformed at birth. Granting in that event that the cretinism caused their degeneration, no postnatal influence, as hyperthyroidism, could possibly call forth their subsequent appearance.

Whether the ovarian atrophy is specifically due to the thyroid deficiency, or to the more general physiological disturbance, secondary to the hypothyroidism, has not been determined. The marked anemia constantly accompanying cretinism in the rabbit should be considered, but certainly cannot be the essential factor in as much as clinical cases of pregnancy have been reported in women with an equal degree of anemia.

Despite the evidence of apparently active ovaries in cretin rabbits fed for a long period, with *large amounts* of thyroids, and the evidence of mating, these animals rarely, if ever, gave birth to young.[3] In order to throw light on the mechanism of this phenomenon, the following experiments were performed: Eighteen female rabbits were fed desiccated thyroids in dosages varying from 90 to 660 mgm. per rabbit per day. Varying amounts were used because of variations in susceptibility to the thyroid substance in different rabbits. In some of the animals severe hyperthyroidism was induced before mating. Others were normal at the time of mating, but in these the thyroid feeding started on the same day of mating.

The evidence for effective mating was obtained. First, by finding sperm on the vaginal smears of isolated females, which had been placed in the mating cage for not more than one hour. Second, by palpation of the fetuses about eight days after observing sperm in the vaginal smears. Third, by performing laparotomy incisions and counting the fetuses on the seventh to the fifteenth day of gestation.

Table 1 contains data showing that in the nine rabbits with laparotomies, the fetuses counted on the seventh day to the fifteenth day of gestation varied in number from nine to fourteen.[4] Only two living young were born of this group: i.e., animals 4 and 10 each gave birth to one living young. Animal 8 gave birth to one well developed dead fetus at term. No. 12 delivered one immature dead fetus before term, nos. 5 and 10 each

[3] A small amount of thyroid, administered daily, is favorable to reproduction in cretin rabbits.

[4] This is a greater number of fetuses than was found by making laparatomies on an equal number of normal rabbits and counting the fetuses.

TABLE 1

Summary of the influence of severe hyperthyroidism on gestation in normal and hypothyroid rabbits

NUMBER OF RABBIT	THYROID FEEDING BEGAN	DATE OF MATING	THYROID FEEDING DISCONTINUED	NUMBER OF DAYS FED DURING GESTATION	VARIATION IN DAILY DOSE	DATE OF LAPAROTOMY (FETUSES COUNTED)	DATE OF DELIVERY (NUMBER AND CONDITION OF FETUSES)
4	2/13/26	4/ 9/26	5/ 8/26	29	Constant dosage 290 mgm.	4/17/26 (9)	5/ 8/26 (1 living)
5	3/30/26	4/ 9/26	5/ 3/26	21	Constant dosage 290 mgm.	4/21/26 (9)	5/11/26 (2 dead)
8	4/ 8/26	4/19/26	5/26/26	14	Constant dosage 430 mgm.	4/29/26 (9)	5/21/26 (1 dead)
10	3/21/26	4/15/26	5/13/26	19	290 to 430 mgm.	4/30/26 (12)	5/15/26 (2 dead, 10 living)
11	3/31/26	4/21/26	5/20/26	22	290 to 430 mgm.	4/30/26 (10)	5/22/26
12	3/14/26	3/23/26	4/14/26	15	Constant dosage 430 mgm.	3/29/26 (14)	4/16/26 (1 dead)
13	3/31/26	4/ 3/26	4/26/26	8	290 to 430 mgm.	4/12/26 (9)	Complete resorption
14	4/ 1/26	4/16/26	4/28/26	10	Constant dosage 430 mgm.	4/23/26 (11)	†
19	4/ 7/26	4/19/26	5/ 7/26	7	Constant dosage 430 mgm.	4/26/26 (14)	‡
25A	4/20/27	4/18/27	5/17/27	25	90 to 660 mgm.	Not performed	Complete resorption
25B	5/27/27	5/25/27	6/16/27	18	60 to 390 mgm.	Not performed	6/27/27 (3 dead)
28	5/27/27	5/25/27	6/22/27	21	60 to 390 mgm.	Not performed	Complete resorption
1B	4/23/27	4/18/27	5/16/27	21	90 to 660 mgm.	Not performed	Complete resorption
2	5/27/27	5/26/27	6/21/27	20	60 to 390 mgm.	Not performed	Complete resorption
3*	4/12/27	4/11/27	5/10/27	24	90 to 330 mgm.	Not performed	5/11/27 (2 dead)
6B	4/16/27	4/11/27	5/11/27	25	90 to 660 mgm.	Not performed	Complete resorption
7*	4/12/27	4/11/27	5/10/27	24	90 to 330 mgm.	Not performed	5/11/27 (2 living, 3 dead)
9	4/12/27	4/11/27	5/11/27	24	90 to 660 mgm.	Not performed	Complete resorption

* These animals were completely thyroidectomized between two and three weeks after birth. After pronounced symptoms of cretinism had developed, and about one-half of the growth period had passed, they were fed small amounts of desiccated thyroids daily with the intent of keeping them approximately normal, until the beginning of pregnancy. They were then fed large amounts of the thyroid substance.

† Animal died 5/13/26. Autopsy revealed resorption of fetuses in progress.

‡ Animal died 5/10/26. Autopsy revealed fetuses almost completely resorbed.

delivered two dead. Animals 14 and 19 died during the last week of gestation, autopsy revealed almost complete resorption of the fetuses.

Laparotomies were not performed on the second group of nine animals, thus ruling out the possibility of the surgical intervention interfering with the development of the fetuses. The evidence obtained from the first group was sufficient to warrant our concluding that severe hyperthyroidism did not interfere with fertilization in the rabbit and that the number of ova fertilized from the mating of a normal male and a female with severe hyperthyroidism was greater than occurred under normal conditions. Positive evidence of pregnancy in the second group consisted in finding sperm on the vaginal smears and palpating fetuses eight to twelve days later. Data found in table 1 show that only one of this group (no. 7) delivered living young. This animal gave birth to two living and three dead rabbits at term. In all others, the fetuses were either completely resorbed or only one to three dead were delivered.

Evidence obtained from the exploratory laparatomy incisions indicates that fetal death and resorption occurred after the first third of the gestation period. It may be that in the later stages of gestation the fetus is more susceptible to the maternal hyperthyroidism, and death essentially from hyperthyroidism followed. Or the stage of development of the fetus when death occurs is merely a coincident, since the severity of the maternal hyperthyroidism probably increased as time progresses, when there is no interruption of the thyroid feeding. Or death of the fetus may be due to abnormal development of the placenta as occurs in pregnant rats, fed on a dietary deficient in vitamin E, Evans et al. (1927).

SUMMARY

An apparent increase in the number of developing Graafian follicles and primordial ova may be demonstrated in the ovaries of cretin rabbits, converted into a condition of severe hyperthyroidism, by the ingestion of thyroid substances.

In rabbits with severe hyperthyroidism experimentally induced, the processes of oestrus, ovulation, fertilization, migration and implantation take place, but in most instances the young are never born, resorption occurring instead. Resorption of all or many of the fetuses occurs during the latter two-thirds of pregnancy.

BIBLIOGRAPHY

Daly, P. A. et al. 1925. Journ. Amer. Med. Assoc., lxxxiv, 1798.
Davis, C. H. 1926. Journ. Amer. Med. Assoc., lxxxvii, 1004.
Guedernatsch, J. F. 1915. This Journal, xxxvi, 370.
Evans, H. M. et al. 1927. Memoirs of the Univ. of Cal., viii.
Hofmeister, F. 1893–94. Beitr. g. Klin. Chir., xi, 441.

HOSKINS, R. J. 1910. This Journal, xxvi, 426.
HOWARD, C. P. 1922. Endocrinology and metabolism. D. Appleton Company.
KUNDE, M. M. AND T. PROUD. 1929. This Journal, lxxxviii, 446.
MINOT, C. S. 1892. Human embryology. William Wood & Company, New York.
MUSSEY, R. D. ET AL. 1926. Journ. Amer. Med. Assoc., lxxxvii, 1009.
SMITH, R. S. 1928. Amer. Journ. Obst. and Gynec., xiv, 518.
TATUM, A. L. 1913. Journ. Exper. Med., xvii, 636.
YOAKUM, W. A. 1928. Amer. Journ. Obs. and Gynec. xv, 617.

STUDIES ON THE BLOOD PLASMA CALCIUM OF NORMAL AND PARATHYROIDECTOMIZED ALBINO RATS[1]

W. R. TWEEDY AND S. B. CHANDLER

From the Department of Physiological Chemistry, and the Department of Anatomy, Loyola University School of Medicine, Chicago, Illinois

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The work herein reported deals with the change which occurs in the total blood calcium of the albino rat, during the first three hundred days following experimentally complete parathyroidectomy, and secondly with the increased sensitivity which such an animal exhibits toward parathormone, during the first few weeks following bilateral parathyroidectomy.

Unilateral parathyroidectomy. Salvesen (1923) reported seven partially parathyroidectomized dogs, which never developed tetany, and whose blood calcium was never below 7 mgm. per 100 cc. of serum, but was usually about 8, and which in each case was restored to a normal value of 10 mgm. or more within a few weeks.

Pepere (1908) found that removal of the external parathyroids in the rabbit produced severe symptoms of parathyroid insufficiency. These symptoms lessened and disappeared following subcutaneous graft of two glands. Even after complete absorption of the glands the animals remained well.

Tanberg (1908) observed that a partial parathyroidectomy in rats or dogs produced a latent, or chronic tetany, and an hypertrophy of any remaining parathyroid remnants.

Piecemeal ablation. In case of the rat (7 males, 6 females, 57 to 91 days of age) we found that the blood plasma calcium varied between 9.25 and 12.5 mgm. per 100 cc., 6 to 57 days following unilateral parathyroidectomy. In four instances (3 males, 1 female) the second gland was removed 18 to 24 days following the first extirpation. Although the blood calcium dropped to 7 to 8 mgm. within 7 days in three of these animals, it regained a normal value within 35 days or less.

Bilateral parathyroidectomy. When bilateral parathyroidectomy was performed at once, it will be observed that the blood plasma calcium very soon dropped to values between 5 and 8 mgm., and had not regained its normal value three hundred days later, except in very few cases. In these

[1] Published in abstract form in Science, 1927, lxvi, 146. Reported before the Pathological Society of the Federation of American Societies for Experimental Biology at Ann Arbor, Michigan, April 12, 1928.

instances incomplete extirpation, or accessory parathyroid tissue may have been partly responsible.

The presence of accessory parathyroids is admittedly a source of error in all parathyroid experimentation. The rat, however, is especially favorable material, as but two parathyroid anlagen develop. Moreover, if two complete glands are removed as shown by serial sections, the percentage of error will be small, for as shown by Hoskins and Chandler (1925), probably less than 10 per cent of all rats have accessory parathyroids. These workers made serial sections of all structures from the level of the lower jaw to the heart of 14 embryos, and 6 newborn animals. No accessory parathyroid tissue was found. Serial sections of the neck region of 6 newborn, and 39 adult animals resulted in the detection of one accessory in each of 5 adult animals.

In view of our observations, we think that some factor other than hypertrophy of accessory parathyroids, was responsible for the greater ease with which normal blood calcium values were regained by animals subjected to piecemeal ablation of all their known parathyroid tissue, as compared to animals subjected to complete parathyroidectomy in one stage.

Animals and diet. The rats used in these studies were descendants of three pairs purchased from the Wistar Institute in 1926. Their diet consisted mainly of an intimate mixture of the following six ingredients, which was supplied daily ad libitum: whole ground wheat (66 per cent), casein (14.5 per cent), Klim (whole milk powder, 10 per cent), sodium chloride (1 per cent), calcium carbonate (1.5 per cent), and butter (7 per cent). In addition lean, cooked meat was supplied once weekly, and lettuce and white bread, twice weekly. Tap water was supplied daily ad libitum.

Technic of parathyroidectomy. The technic of parathyroidectomy employed has been used by one of us (S. B. C.) for four years with but little modification. The tissue removed is in every instance sectioned, stained and examined microscopically. No operation is considered complete, unless the two glands removed are of approximately the same size, surrounded by a connective tissue capsule, or by thyroid tissue, and free from sharp borders, such as would be produced by incomplete extirpation.

Blood samples. The blood samples were taken, by cardiac puncture, while the animals were under light ether anesthesia. The animals were subjected to a preliminary starvation period in only a few instances. The blood was rendered non-coagulable by heparin, and the analyses, in case of both normal and parathyroidectomized animals were in practically all instances made upon 1 cc. amounts of plasma. This procedure was adopted in order to conserve the animal's blood, and in order to keep the results more nearly comparable. As a further means of economizing blood, the plasma, after centrifugation of the blood at high speed was with-

drawn by suction applied through a rubber tube, containing a bead, to which was attached a 1 cc. pipette, graduated in hundredths of 1 cc. to the tip.

Method of calcium analysis. The Kramer-Tisdall (1921) method as modified by Tweedy and Koch (1926) has been used in these studies. It differs from the unmodified method, in that loss of the precipitated calcium oxalate, through solubility in the subsequent washings with 0.5 per cent NH_4OH is prevented, by using 0.5 per cent NH_4OH saturated with CaC_2O_4. It differs from the Clark-Collip (1925) modification in the same respect, and also in that the latter modification relies upon only one washing with 0.5 per cent NH_4OH for removal of contaminating ammonium and magnesium oxalates, while three washings are here used. The method was

TABLE 1

Blood plasma calcium following bilateral parathyroidectomy

NUMBER OF RATS	TIME AFTER OPERATION	BLOOD PLASMA CALCIUM		
		Lowest	Highest	Average
	days	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
6	1	5.01	8.72	7.30
4	2	5.20	10.10	7.41
5	3	7.32	10.59	9.01
5	4	5.35	8.86	7.64
1	5			7.50
4	6	8.28	9.14	8.71
3	8–13	4.81	6.83	6.05
7	30–50	4.93	10.00	6.83
9	68–100	4.32	9.98	7.41
9	113–145	5.39	9.24	7.44
12	200–311	5.81	12.06	8.60

further rendered more sensitive, and the analysis made more convenient by using the Koch (1926) micro burette.

Blood plasma calcium after bilateral parathyroidectomy. It is apparent from our data, obtained over a period of 300 days following bilateral parathyroidectomy, that the very definite lowering of the blood plasma calcium, obtained within 24 to 96 hours, was followed by an interval of some 100 days, or more, during which the calcium tended toward even lower values. Following its probable lowest level, attained during this period, a very definite tendency toward higher values is manifested, and a return to just below a low normal, or a normal value is usual by the end of 200 to 300 days. It will be noted that only 6 animals of the 65 studied, were found to have attained a normal blood calcium. Since the blood samples were withdrawn from the animals without a preliminary period of starvation, it

seems reasonable to assume that the basal value, so obtained, may have been somewhat lower in every instance than the values here given.

The two animals in group 2 which showed high blood calcium were found to have attained values between 5 and 6 mgm. three days later. Only one of four animals selected from the four succeeding groups and subjected to three additional blood calcium tests had attained a normal value at the end of 200 days. It may be further stated that no relation of age or sex to change in blood calcium after parathyroidectomy has been noted.

Sensitivity to parathormone. Our observations on the state of the blood plasma calcium following parathyroidectomy has not demonstrated any definite relationship of the low calcium values found to active tetany. However, the condition of the animals in other respects demonstrated that they were abnormal and that the condition of acute parathyroid insufficiency was compensated for very gradually.

Very few data have been submitted concerning the effect of parathyroidectomy upon sensitivity to the calcium raising effect of parathyroid hormone. Collip and Clark (1925) reported that the calcium raising effect of parathormone is practically the same in the thyroparathyroidectomized dog as in the normal dog. It is not evident from their paper, however, that they have considered time after parathyroidectomy as a possible factor affecting sensitivity. A. M. Hanson (1928) found that while there is a great variability in the response of normal dogs to the hormone, there is a marked uniformity in the response of thyroparathyroidectomized dogs.

Our studies indicate that during the first 25 days following parathyroidectomy the rat is 2 to 3 times more sensitive to the calcium raising effect of the hormone. As yet we have not attempted to establish the duration of this altered response.

Parathyroid extracts. It will be noted that two types of parathyroid extract have been used.

The preparation indicated, Parathormone (Lilly), was obtained from Eli Lilly and Company and contained about 1 per cent of solids per 5 cc., or for each 100 units. The preparations designated P15, etc., were prepared by a modification of the senior author's (Tweedy, 1926) method. Just prior to injection the given amounts were dissolved in a few cubic centimeters of physiological salt solution, and administered either subcutaneously, or intraperitoneally. Seventeen to twenty hours later blood was withdrawn and the calcium content determined.

Crude hydrochloric acid extracts of rat parathyroids were also in two instances injected. While no definite conclusion can be drawn by one such comparison, the results suggest that the calcium raising principle may be most specific for the species which elaborates it. Furthermore it may be

TABLE 2

Administration of parathyroid extract to parathyroidectomized rats

RAT	AGE	WEIGHT	BLOOD PLASMA CALCIUM	TIME AFTER PARATHYROIDECTOMY	REMARKS
	days	*grams*	*mgm. per 100 cc.*	*days*	
1	90		8.28	20	
				21	Received 15 mgm. P16 intraperitoneally
			17.75	22	
			10.94	25	Died immediately after cardiac puncture
2	144	193		25	Received crude HCl extract of 40 rat parathyroids
			13.34	26	
			9.06	31	
			5.77	146	
				151	Received 10 mgm. P19 subcutaneously
				152	Received 10 mgm. P19 subcutaneously
			8.35	153	
			5.49	158	
		267	7.67	272	
3	144	199		25	Received 9 mgm. P19 subcutaneously
			11.98	26	
			7.80	31	
				36	Received 15 mgm. P10 subcutaneously
				37	Received 15 mgm. P10 subcutaneously
			20.00	38	
			6.36	116	
4	150	150	5.40	7	
				14	Received 100 units parathormone (Lilly) intraperitoneally
				15	Same quantity
			18.98	16	
			7.74	30	Killed
5	150	254	4.40	7	
				14	Received 100 units parathormone (Lilly) intraperitoneally
			15.32	15	
6	42	137	7.12	43	
				50–52	Received 10 mgm. of P19 subcutaneously daily
				53	Received 15 mgm. of P19 subcutaneously
			13.61	54	
			6.26	57	
				70	Received 100 units parathormone (Lilly) subcutaneously
			9.84	71	

TABLE 2—*Concluded*

RAT	AGE	WEIGHT	BLOOD PLASMA CALCIUM	TIME AFTER PARATHYROIDECTOMY	REMARKS
	days	*grams*	*mgm. per 100 cc.*	*days*	
7	56	104	4.81	12	
				25–29	Received daily 15 mgm. P20 intraperitoneally
			11.93	30	
				31	Received 7 mgm. intraperitoneally
				32	Received 15 mgm. intraperitoneally
			9.05	33	Died immediately following heart puncture

that the ease of separation of the active principle from extraneous material will vary with the glandular material employed.

Parathormone overdosage. The dog has been found to be peculiarly sensitive to injections of the parathyroid hormone (Collip, Clark and Scott, 1925; Collip and Clark 1925). According to these authors and others (Matthews and Austin, 1927) the characteristic finding at death is intense hyperemia and venous engorgement of the stomach and intestines. Considerable blood is also usually found in the stomach and intestines.

Macroscopic examination of the viscera of normal and parathyroidectomized rats, which had apparently died of hormone overdosage, failed to reveal any such condition as that described above. The stomach and intestines of these animals were normal in appearance.

Reaction of normal rats to parathyroid extract. Two rats (av. weight 128 grams) received, either subcutaneously or intraperitoneally, 15 mgm. of P20. Seventeen hours later the blood plasma calcium values were 14.45 and 14.68 mgm. respectively. Four rats (av. weight 174 grams) received 30 mgm. of the same preparation, in two equal doses 17 to 20 hours apart. Approximately 17 hours later the average blood calcium of this group was 13.75 mgm. and the maximum value was 16.18 mgm.

Two rats (av. weight 147 grams) received 100 units (or 50 mgm.) of parathormone (Lilly) in the same manner as the above animals. Approximately 17 hours later their respective blood plasma calcium values were 9.25 mgm. and 14.21 mgm. respectively. Four rats (av. weight 194 grams) received 200 units of parathormone (Lilly) in two equal doses of 100 units each, 17 hours apart. Approximately 17 hours after the last injection the blood plasma calcium of two of the animals was found to be 15.21 and 17.91 mgm. respectively. The other two animals of this group were found dead, so no blood sample following the second injection was taken.

SUMMARY

The blood plasma calcium of non-fasting albino rats maintained on the diet described, usually varied between 9.25 and 12.5 mgm. per 100 cc.

Unilateral parathyroidectomy did not appreciably change this value. Upon removal of the second parathyroid a temporary drop below a low normal occurred, but persisted for only a few weeks.

Following bilateral parathyroidectomy a rapid drop in blood plasma calcium to values between 5 and 8 mgm. per 100 cc. may occur, within 24 hours, or it may decrease more slowly, requiring from 1 to 10 days to attain such values. A definite tendency for the calcium to rise is manifested by the end of 100 days, and by the end of 200 to 300 days it may have attained a value just below a low normal.

The reaction of normal and parathyroidectomized rats to comparable amounts of parathyroid hormone has shown the latter to be two to three times more reactive.

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BIBLIOGRAPHY

COLLIP, J. B., E. P. CLARK AND J. W. SCOTT. 1925. Journ. Biol. Chem., lxiii, 439.
COLLIP, J. B. AND E. P. CLARK. 1925. Journ. Biol. Chem., lxiv, 485.
CLARK, E. P. AND J. B. COLLIP. 1925. Journ. Biol. Chem., lxiii, 461.
HANSON, A. M. 1928. Journ. Amer. Med. Assoc., xc, 747.
HOSKINS, M. M. AND S. B. CHANDLER. 1925. Anat. Record., xxx, 95.
KOCH, F. C. 1926. Journ. Lab. Clin. Med., xi, 774.
KRAMER, B. AND F. F. TISDALL. 1921. Journ. Biol. Chem., xlvii, 475.
MATTHEWS, S. A. AND W. C. AUSTIN. 1927. This Journal, lxxix, 708.
PEPERE, A. 1908. Arch. d. med. exper. Anat. Path., xlix, 336.
SALVESEN, H. A. 1923. Journ. Biol. Chem., lvi, 443.
TANBERG, A. 1914. Mitt. a. d. Grenzgeb. Med. u. Cher., xxvii, 575.
TWEEDY, W. R. AND F. C. KOCH. Journ. Lab. Clin. Med. (in press).
TWEEDY, W. R. 1926. Proc. Soc. Exper. Biol. Med., xxiv, 147.
TWEEDY, W. R. AND S. B. CHANDLER. 1927. Science, lxvi, 146.

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